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8) ABSTRACT The medical consequences of sustained military operations have been studied in cadets from the Norwegian Military Academy during military training courses with physical activities day and night combined with lack of sleep and food. The increased catecholamine responses to a standardize exercise, downregulation of ß-receptors and decreased cAMP responses to adrenaline stimulation indicate an adrenergic desensitization. Conjugated catecholamines increased during prolonged exercise but marginally during short term exercise. The increased catecholamine levels during the course were mainly due to physical exercise, whereas alterations in cortisol and growth hormone were partly reversed by extra food, and extra sleep did not have any major influence. Both testicular and adrenal androgens decreased during the course. The decreased levels of gonadotropins and their increased responses to hypothalamic releasing hormone indicate that the decreased secretion of testicular androgens is due to decreased hypothalamic secretion of releasing hormone. The circadian rhythm of all steroid hormones was extinguished during the course in contrast to the increased amplitude of the circadian rhythm of mental performance. In conclusion, these alterations may affect functions which are the basis for the subjects' mental and physical performance. Some of these alterations are necessary adaptations in order to maintain mental and physical performance capacity, whereas other alterations may impair performance, be harmful and produce diseases which should be avoided or treated

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PREFACE

The present work is part of a more extended research program that originally started in the Norwegian Joint Medical Services approximately 20 years ago. The idea of stress research was conceived among Norwegian physicians with experience from the second world war. Major General Alf A. Johnsen, who was a member of the Linge company, was the formal leader, and Arve Lønnum, who had studied the long term health consequences of combat stress, was the scientific leader. Other members of the group were Odd Øyen, Ole Jacob Malm, Karl Reichelt, Arne Sund and Frode Fonnum. Kåre Rodahl, with his large experience in applied medical and occupational research, was the first to see the ranger course at the Norwegian Military Academy as a suitable model for military stress research. His enthusiastic support and interest is greatly appreciated. I am grateful to all the cadets who have enthusiastically participated in my different research projects, and to the Norwegian Military Academy, its officers and leaders for excellent co-operation.

With his interests in basic neurochemical research, and endocrinology and metabolism as "applied neuroscience", Frode Fonnum established stress and endocrinology as an area of research at the Norwegian Defence Research Establishment (NDRE) and provided research facilities with support form the director, Finn Lied, and later endorsed by Erik Klippenberg and Nils Holme. I am thankful to Jon Storm-Mathisen who taught me the art of writing my first scientific paper, and to Asbjørn Aakvaag for his collaboration during my first steps in endocrine and metabolic research. I also want to thank Olav Øktedalen, Pål Wiik, Jan Fredrik Bugge, Magne Bråtveit and Arne Bøyum for their collaboration, and Erling Seeberg, Ivar Walaas, Sigrunn Sterri, Jan Blanch, Ragnhild Paulsen, Bjørnar Hassel and Dagfinn Løvhaug for discussions. Berit Andersen, Liv Eliassen and Ann-Helen Haugen have given skilful technical assistance. Most of all I am grateful to my excellent reviewer, Knut Kristian Skrede.

Kjeller, December 1995

Per-Kristian Opstad

To my parents

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Paper IV Opstad PK, Wiik P, Haugen AH, Skrede KK (1994). Adrenaline stimulated cyclic adenosine monophosphate response in leucocytes is reduced after prolonged physical activity combined with sleep and energy deprivation. Eur J Appl Physiol **69**: 371-375.

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Paper VII Opstad PK (1994). Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep and energy deficiency in young men. Eur J Endocrinol **131**: 56-66.

Medical Consequences in Young Men of Prolonged Physical Stress with Sleep and Energy Deficiency

1 GENERAL INTRODUCTION

1.1 The concept of stress

The word stress originates from physics, the sciences of metallurgy, and is defined as the power, strain or straining force exerted upon a body that tends to deform its shape. It is usually measured in pounds per square inch (Guralnik 1982).

The concept of stress was introduced in the biomedical and human sciences by the Canadian physiologist Hans Selye approximately 50 years ago. He divided the stress reaction into 3 separate stages: an "alarm reaction" with an activation of the sympatho-adrenal system, a subsequent "stage of resistance" with activation of the hypothalamo-pituitary axis, and a final stage with exhaustion and death. The "stage of resistance" included his "general adaptation syndrome", and his "disease of adaptation". He defined stress as a non-specific response of the body to any demand made upon it and his stress concept became more or less synonymous with activation of the hypothalamo-pituitary-adrenal axis and the adrenal secretion of cortisol (Selye 1936, 1946, 1950, 1970, 1978).

Before Selye the French physiologist Claude Bernard described " le milieu interieur" (Bernard 1879) which was considered essential for normal functioning and for the maintenance of life. Cessation of blood circulation for only a few minutes will cause irreversible damages or death. The concentration of the different components of the blood is kept within physiologically appropriate limits by effective regulatory devices.

Cannon introduced the term "homoeostasis" for the maintenance of this constant state and its regulation (Cannon 1929, Adolph 1961). When this state is "disturbed" by external or environmental factors the body always tends to re-establish the equilibrium of this internal environment.

However, the first to describe the relationship between the organism and its environment was Darwin in his concepts of natural selection. In his view, the environment changes constantly (seasonal, climatic, chemical, geological etc.), or it is being changed by its inhabitants. The "stress" of the environment is a selective pressure that can be threatening to the survival, integrity and reproductive success of individual and species. The environmental challenges, often elicit physiological and behavioural responses which are specific and appropriate to the stressful situation, to prevent sickness and death.

After the discovery of the catecholamines Cannon (1929) later also introduced the concept of activation, which means that the body has to be activated (for example by the secretion of catecholamines) in order to perform physically or mentally. This activation has also been expressed in the terms of “fight or flight” which means that the subject has to be activated in order to be fit for “fight or flight”. Selyes non-specific response to stress can partially be explained by the contemporary limited laboratory facilities to analyze biological stress parameters such as hormones and peptides.

During the last years it has become apparent that several important aspects are missing in Selyes definition of stress. The first is that specific stressors do produce specific patterns of hormonal and neurochemical responses to different challenges or situations, which depend on several factors including the ability to cope with the stress (weather the stress is escapable or inescapable) (Weiss 1971, Anisman et al 1980), the physical and psychological consequences of stress (Martin 1984, Gibbs 1986), and the social conditions (Thoa et al 1977, Holahan and Moos 1985), age (Ritter and Pelzer 1978), and genetic constituents of the individuals (Wimer et al 1974, Gottschalk 1983). Another aspect is that animals for instance have a variety of behavioural strategies for survival in dealing with the threat of predators. These behavioural responses must be appropriate to the threat if they are to succeed. If they were random or indiscriminate (non-specific), survival would not have been assured. A third aspect is that even if the physiological responses to stressors are with some exceptions similar between species and genera, it is the interpretation of the threatening signals that differs between different species. It is not the physical form of the signal but rather its value as a "predictor or correlate" that is important (Kagan and Levi 1971, Levins and Lowontin 1985, Yirmiya et al 1991).

It is assumed that both immune and endocrine responses to stress are dampened by defence and coping. These two mechanisms have different time axis. Coping is thought to be related to the fast catecholamine response, whereas defence is assumed to be related to the slower pituitary-adrenal response. The role of the cortisol response might be to suppress and dampen this acute stress response in the later phase. Low health risk depends on the stress-dampening mechanism of cortisol (Sutherland and Cooper 1990, Henry 1992, Levine 1993, Ursin and Olff 1993, Munck and Náray-Fejes-Tóth 1995).

Gradually the concept of stress has evolved from non-specific to rather specific and appropriate responses to the different stressors that the body is exposed to. These stressors are often environmental challenges such as sleep deprivation, physical exercise, energy deficiency, time pressure, cold exposure, heat exposure etc, to which the body's responses might be modulated by personality structures, coping, knowledge, experience etc. Disease, military attrition, irrational stress responses occur when defence or coping mechanisms are maladapted to face the subjects’ problems or also when the energy required from the body to perform a task is unreasonably high.

1.2 The multifactorial stress

The body is often exposed to combinations of different stressors. This is always the case in military field operations where the stressors often are prolonged, hard, continuous physical exercise combined with sleep, energy and water deficiency, cold, heat, time pressure or also boring endless waiting (Bourne 1969, Johnson and Naitoh 1974, Opstad et al 1978, 1984, 1985, Opstad and Aakvaag 1981, 1982, 1983, 1983, 1984, Angus et al 1987, Moore et al 1992, Friedl et al 1993, 1994, Belenky et al 1994, Guezennec et al 1994, Johnson et al 1994, Shippee et al 1994). The interaction between different stress factors is in a multifactorial stress-situation, however, scarcely investigated.

Many researchers prefer to work with pure models under well controlled conditions and preferably in a laboratory environment. However, the interaction between different stress factors can not be predicted from knowledge of effects of each single stress factor alone. The different stress factors may act in concert or synergy, but they may sometimes also have opposite effects and counteract one another. This is a main reason why the final results only can be achieved in multifactorial field studies. For almost all practical purposes it is the multifactorial field situation which is the most relevant, and decrement of performance may be postponed with a precise knowledge of the stress factors crucial for the type of performance needed to fulfil a certain task.

Also in intensive care units, most patients are subjected to a multitude of stress factors, particularly so for the multitraumatized patients (Souba and Wilmore 1988, Kinney and Tucker 1992, 1994). The interpretation of the results from such studies may, however, be complicated by the patients' treatment and medication and the fact that appropriate control groups may be lacking.

The significance of pre-exposure to different stress factors for the recovery and treatment of the multitraumatized patient is still an open area for research. It is unclear if pre-exposure to strenuous exercise with its catabolic metabolism with high priority for energy production will hamper recovery and survival possibility for the multitraumatized patient, or whether, on the contrary, the very strong anabolic response following such strain will improve restitution of the patient. Adequate nutrition supply and/or substitution therapy may also be beneficial for survival of multitraumatized patients and shorten their recovery period.

1.3 Physical exercise and exhaustion

Physical training increases the human work capacity by increasing the number and size of mitochondria and particularly the mitochondrial aerobic enzymes. The aerobic capacity may be doubled by training such as running. During prolonged exercise physical performance is limited by the maximal oxygen transport capacity which mainly depends on the maximal capacity of the heart. In contrast the anaerobic enzymes show much more

modest alterations (Hultman et al 1988, McArdle et al 1991, Saltin and Strange 1992, Vogel 1994, Galbo 1995). From rest to maximal exercise the energy production may increase 300 fold. The immediate energy source, adenosine triphosphate (ATP), is found in the muscle in small amounts (5.5 mmol/kg muscle) and is continually synthesized and resynthesized in the mitochondria. This synthesis depends on the size, number, and enzymatic content of the mitochondria as well as the rate of oxygen uptake and transport. These energy stores are rapidly depleted during exercise. The substrates for resynthesis of ATP are glucose, glycogen stored in the muscle and free fatty acids, in addition to small amounts of triglycerid depots stored within the muscle cells. However, the anaerobic combustion of glucose gives only 3 ATP, whereas aerobic combustion gives 38 - 39 ATP per glucose unit when oxidized, which means that during prolonged exercise only aerobic combustion will give significant amounts of energy. At low exercise intensities the muscle prefers to use fat for the generation of ATP, but with increasing exercise intensities the proportion of carbohydrate combustion increases (McArdle et al 1991, Saltin and Åstrand 1993).

During a 5 day ranger training course at the Norwegian Military Academy the cadets' physical activities average 35 % of VO_2 max with a mean pulse rate of 111 beats/min. This low exercise intensity represents mainly fat oxidation. However, figure 1 shows that this intensity may change from a pulse rate of 50 to 150, and only rarely to above 150 beats /min, and this changing work intensity might increase the need for carbohydrate consumption (Waldum and Huser 1974, Aakvaag et al 1978, Rognum et al 1982). The resting heart rate increased with only 9 beats /min during the course and during some courses a slight increase has been seen for the pulse rate response to a standard bicycle exercise test. The resting R-value in the control experiment was 0.78 ± 0.02 indicating a combustion of 26 % carbohydrate and 74 % fat. This R-value was reduced during the course to 0.65 ± 0.01. Since a pure fat combustion gives an R-value of 0.7 the subjects are not only on a "pure" fat combustion, but in addition they have a considerable oxygen debt that has to be restored. However, when the subjects are tested on the bicycle test and thereby increase their energy production, there is also an increase in the R-value to 0.77 ± 0.01, which indicates a carbohydrate portion of at least 23 %, whereas in the control experiment the R-value increases to 0.86 ± 0.01, indicating a carbohydrate consumption of 54 %. Glucose infusion during the exercise test gave 2 mmol/l increase in the plasma levels in both experiments. This shows that the plasma glucose level does not in itself influence glucose combustion. Surprisingly, even after several days without carbohydrate supply, the cadets do not profit from the carbohydrate infusion by increasing carbohydrate combustion to any measurable extent. In stead of increasing the muscle uptake of carbohydrates, most of the infused glucose rest in plasma, resulting in a state of hyperglycaemia which might hamper both mental and physical performance (Bahr et al 1991).

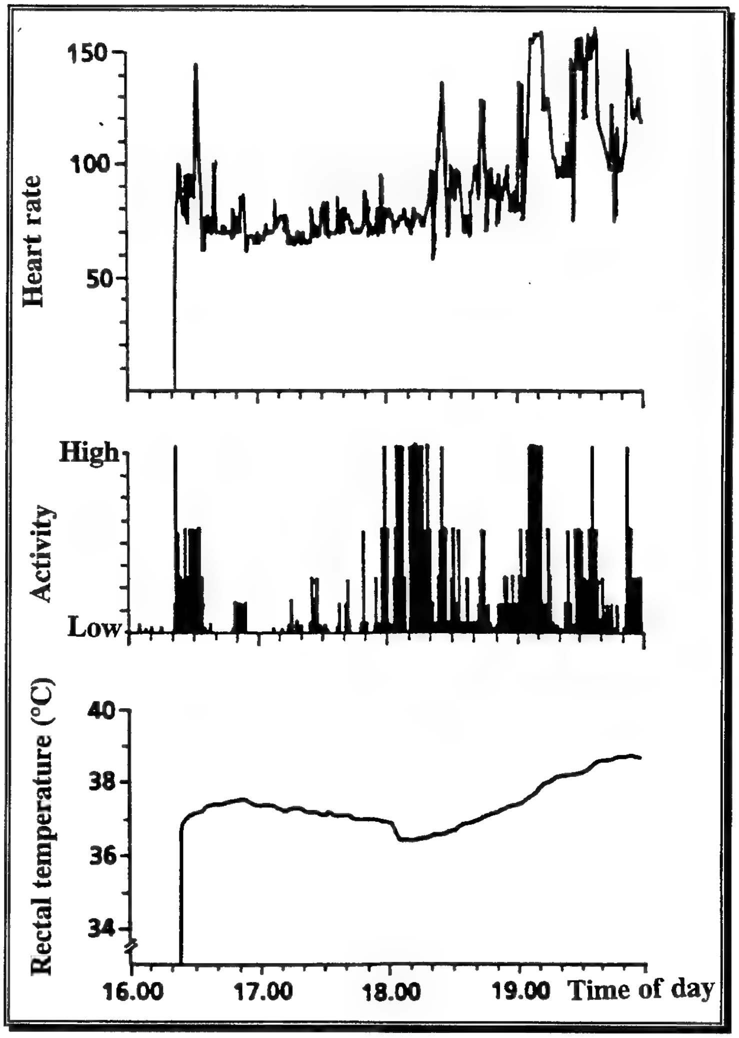

Figure 1.1 Alterations in heart rate, left wrist activity and rectal temperature (°C) (Vitalog recording) from one cadet on day 2 during a 5 day military training course at the Norwegian Military Academy.

It is also shown that the oxygen consumption is increased by 14-24 % both at rest, during a standardized exercise test and during postexercise recovery. The mechanical efficiency of the muscles deteriorate from 24.6 % in the control experiment to 20.9 % during the course (Bahr et al 1991).

A state of exhaustion means that the subjects' physical work capacity is strongly reduced and that health or life itself may be at risk. Several different mechanisms may lead to this state of physical exhaustion. First of all the energy reserves in the muscle are strongly reduced or empty, this concerns mainly the glycogen reserves which are limited (300-400 g). This state is often observed in athletes who suddenly have a decrease in their physical performance during prolonged physical competition. Fat is, however, still an energy source which allows physical activities for several weeks but at a lower intensity level. Another reason for impaired physical performance is painful overused muscles which the subjects avoid to use (Armstrong 1984, Kuipers 1994). This may often be the case during the ranger

training course, particularly for those who are rather untrained at the start of the course. Another explanation may be impaired nervous control of the muscle movements, weakness, lack of co-ordination and balance disturbances. Alterations in the Na/K pump and other homeostatic mechanisms may also affect muscle contraction, however, this does not seem to be the case during the ranger training course (Opstad et al 1985).

1.4 Consequences of lack of food and starvation

Fat is the major fuel storage for humans and animals. Normally fat represents 20 - 25 % of body weight and would provide fuel for 60 - 70 days, assuming an energy consumption of 2000 kcal/day. An obese individual is reported to survive a fast of 310 days (Barnard et al 1969), whereas the survival of nonobese subjects coincides roughly with the predicted depletion time of fat, approximately 60 days (Leiter and Marliss 1980). The cadets in the present investigation are well trained and have lower fat stores than the above mentioned normals, namely approximately 15 - 20 % of their body weight. However, for some of the cadets the fat stores may be down to 10 %. It is assumed that if fat stores are reduced to below 8 % of body weight, vital, essential or structural fat particularly in the cell membranes, brain and nerve sheets is affected, which may therefore reduce mental or physical performance or be detrimental for the subjects' health (Munro 1964, Grant 1983, Hoffer 1988, Moreley and Silver 1991, Johnson et al 1994, Moore et al 1992, 1993, Friedl et al 1993, 1994, Shippee et al 1994, Heber 1995, DeFronzo and Ferrannini 1995).

In contrast to fat, carbohydrate is a quantitatively insignificant storage fuel. Carbohydrate is stored as glycogen in muscle (300 - 400 g) and in the liver (70 g). In addition circulating blood glucose represents approximately 20 g. The carbohydrate stores therefore represent approximately 2000 kcal which is less than one day's resting energy consumption. However, glucose has unique characteristics that makes it an essential body fuel. It can be metabolized along a glycolytic pathway without the use of oxygen, it is rapidly mobilized and is the main fuel generating $NADPH_2$ and is indispensable for several metabolic processes, particularly in the brain, blood cells, kidney and liver. Unfortunately carbohydrate stores contain little energy per weight and volume compared to fat, which may be the main reason for the small reserves in the body. Due to these small carbohydrate reserves a considerable metabolic alteration has to take place during starvation. To economize with the carbohydrate stores, ketones and fat gradually take over as energy source. Ketones (mainly acetoacetate and β-OH butyrate) are synthesized in the liver from fat. It is assumed that ketones can replace 50 % of carbohydrates as energy source during starvation. This may reduce the need for carbohydrates to 100 g of glucose/day mainly for the red blood cells, renal cortex and the brain. During fasting not only the astrocytes and oligodendroglia cells, but also the neurones may replace glucose in the energy production by ketone bodies, lactate, glycerol and pyruvate (Cremer 1971, Berl 1973, Hassel et al 1995). Astrocytes may also β-oxidize fatty acids for energy production (Edmond et al 1987). The importance of ketone bodies is also supported by the finding that fasting prior

to transient cerebral ischemia reduces delayed neuronal necrosis (Marie et al 1990). Ketones are preferred before glucose as precursor for brain lipogenesis for the nerve sheets and membranes (Lopes-Cardozo et al 1986). It is also shown that there are regional differences in the brain as to the combustion of ketones, with the highest consumption in the telencephalon including the hippocampus (Hawkins et al 1986), and that the blood-brain barrier is the rate limiting step for the brain ketone metabolism (Hawkins and Biebuyck 1979). The permeability of the blood-brain barrier for ketones is increased by starvation and diabetes (Gjedde and Crone 1975). However, in spite of the fact that ketones can produce high energy phosphate, they can not maintain neural activity as glucose does (Arakawa et al 1991).

The muscles with the type II fibres prefer to burn fat, whereas the fast twitch fibres are more dependent on carbohydrate combustion. A study of Ahlborg et al (1974) showed that 4 h exercise at a work load of 30 % of VO_2 max resulted in an energy contribution from fat of 37 % during the first 40 minutes, and this increased to 62 % during the last hour. During the first 2-3 days of fasting ketones produced in the liver will replace glucose as energy source also in the muscles. During fasting or in endurance low intensity training, the tissues will increase their fat combustion capacity by increasing the size of the mitochondria and by stimulating the synthesis of their oxydative enzymes. This will lead to a gradually increasing significance for fat combustion during prolonged fasting (for review see: Hoffer 1988, DeFronzo and Ferrannini 1995, Heber 1995).

Since the carbohydrate need can not be reduced to below 100 g/day, this glucose has to be provided from gluconeogenesis. The main substrate for gluconeogenesis is lactate, pyruvate, alanine and glutamine mainly from skeletal muscles and glycerol from the lysis of triglycerids in fat tissue. Gluconeogenesis mainly takes place in the liver, however, after 2-3 weeks of fasting 50 % of the gluconeogenesis takes place in the kidneys with glutamine as a substrate. The relative contribution of glycerol as a precursor may increase by low intensity physical exercise which will stimulate lipolysis and muscular fat combustion.

The energy value of the normal diet used by the cadets of the Norwegian ranger training course is approximately 10 - 15 % proteins, 20 - 30 % fat and 50 - 60 % carbohydrates. According to Dietary Allowances and Food and Nutrition Board (RDA) there is no need for extra protein intake when energy output is increased during training or competition or even during heavy exercise. However, the protein loss during prolonged heavy exercise has been estimated to be approximately 4 % (Lemon and Mullin 1980, Rennie et al 1981, Greenwood 1994) of the total energy expenditure when performed with adequate amounts of glycogen in the muscle and liver, but increases to 10 % when these stores are depleted. Marable et al (1979) has shown an increase in lean body mass of 2 kg after 28 days of heavy weight training with a protein intake of 0.8 g/kg/day. An intake of 2.4 g/kg/day resulted only in a large increase in urinary nitrogen loss but the same rate of body protein synthesis. Many athletes have tried to increase their muscle mass and strength by

combining training with an increased portion of proteins in their diet (as much as 100 g/day) without any significant effect except an increase of urinary excretion of urea followed by an extra need for water. The amounts of dietary proteins exceeding approximately 10 g/day are transformed to energy. Therefore, when the glycogen stores are depleted after some hours of starvation, depending on the intensities of physical activities, there is no protein store that can be used for energy production other than the active tissue proteins. Body proteins are present in the amounts of about 12 kg (dry weight), however, only 6-7 kg of this is in actively metabolizing tissues, the rest being structural. The active protein tissue, which represents a weight of 30-40 kg of hydrated lean tissue, could theoretically supply for a calorie consumption of 2 - 3 weeks at an energy consumption of 2000 kcal/24h. However, in contrast to fat and carbohydrates, even modest depletion of the active body protein content might have profound adverse functional effects. In nonobese subjects, weight loss in chronic starvation is roughly proportional to the lean tissue loss, and protein losses in the range of 50 % or more are incompatible with persistence of life (Hoffer 1988, Hultman et al 1988, Heber 1995, Warren 1995). In the course of fatal starvation in animals, fat loss is accompanied by a continuing depletion of body proteins, although the body tries to save proteins as long as possible on the expense of fat combustion and to reduce the need for energy to a minimum. When the fat stores are depleted, urinary nitrogen excretion rises abruptly and is shortly followed by death. However, since plasma proteins such as immune globulins also decrease at this terminal stage, there is, in addition to oedema, also an increased tendency to infections such as pneumonia, cellulitis, lymphangitis, sepsis etc. which most often is the cause of death before the fat reserves are completely depleted (Garrow et al 1965, Grant 1983, Souba and Wilmore 1988, Moore et al 1992, Shippee et al 1994, Friedl et al 1994).

During the Norwegian ranger training course there is a dramatic decrease in body weight in short time (8 to 12 kg) if the cadets are not given any food, and there are clinical signs of three metabolic phases, however, with a continuous transition between the different phases: The first phase is during the first day and night of activities, when the body is dependent on a relatively high portion of carbohydrate combustion. During the second phase there is a dramatic increase in ketone bodies which are able to partially replace carbohydrates as energy source, also in muscle tissue. However, since only 50 % of the carbohydrate store can be replaced by ketone and fat combustion, the body is still dependent on gluconeogenesis with proteins and glycerol as substrates. The second phase is often very strenuous with considerable physical and psychological discomfort, dysphoria and gastrointestinal problems such as pain and dyspepsia. Some of these symptoms may be due to the high concentration in blood of ketone bodies which are known to cause euphoria at high concentrations. The third phase appears after approximately 3 days, with an increase in the relative portion of fat consumption and a corresponding decrease in ketone oxidation and production, and with a psychologically more relaxed, indolent attitude (Rognum et al 1981). Small amounts of carbohydrates will dramatically reduce this exposure of the body to metabolic stress, and increase the soldiers endurance capacity and economize with the

energy reserves. A diet containing 100 g of carbohydrate each day will prevent the combustion of structural proteins. A further increase will reduce the need for ketone formation, which also will economize with energy reserves. During the ranger course there is a dramatic difference between cadets receiving a daily ration of 4000-6000 KJ/day (70 to 80 % as carbohydrates), who loose about 4 kg body weight during the course, and those who are subjected to total fasting and loose approximately 8-12 kg body weight during the course. Hunger is felt during the course only if the cadets are told that they will have something to eat, which then is well in accordance with Pavlovian theories. Extra food, beyond approximately 1000 to 1500 kcal/day, does not seem to have any major influence on mental or physical performance during this 4-5 day ranger course (Rognum et al 1986). This might be explained by the dominating effect on mental performance of sleep deprivation compared to food deficiency, and that the task tested was short-lasting and of low intensity, and finally that the consequences of energy deficiency normally only appear after more prolonged periods with energy deficiency. It is, however, claimed that not only the amount of food but also the composition of the food may have significance for both mental and physical performance (for review see Ahlers et al 1994, Borum 1994, Greenwood 1994, Ivy 1994, Jandacek 1994, Lieberman 1994, Spring et al 1994, Wurtman 1994, Zeisel 1994). The reduced food intake also results in a decreased volume of the stomach. If the cadets are told that they are going to have dinner, they are hungry, but their stomach is full after half of their normal meal. The first day of recovery the cadets eat small and frequent meals containing more carbohydrate than normal. The cadets are advised not to consume big carbohydrate meals due to their impaired glucose tolerance. The meal size and frequency normalize gradually within 1-2 weeks.

1.5 Temperature regulation during stress, cold or heat exposure

An adequate temperature regulation is essential for mental function and physical performance. Deep body temperature in humans is 37 ± 0.5 ° C with a maximum level in the afternoon and a minimum level during the early morning hours. The variations during the 24 h cycle may be ± 0.5 to 1.0 ° C. Since only 20-30 % of the muscle energy consumption is transformed to mechanical work, 70-80 % is transformed to heat. At rest the total energy production is 70-100 watts. This heat production can be increased at least 10 times by physical exercise for short periods of time. Heat balance can be described by the following formula: $\pm S = M + W \pm R \pm C \pm K \pm E$ where S is gain or loss of body heat, M is metabolic heat production, W is heat production induced by work, R is irradiation heat, C is connective heat transport (to the air), K is conduction to the ground, E is energy loss or gain through evaporation (LeBlanc 1975, Åstrand and Rodahl 1986, Armstrong and Pandolf 1988, Hamlet 1988, Hubbard and Armstrong 1988, Sawka and Wenger 1988, Toner and McArdle 1988, Wenger 1988, Young 1988). During the ranger training course the energy consumption is increased by an average of 4 times compared to normal, which also means at least a similar increase in the heat production.

Only small deviations from normal deep body temperature will affect both physical and mental performance. Deep body temperature is maintained within narrow limits by means of the blood circulation, mainly to the skin and extremities. This circulation is regulated from the hypothalamus, which receives temperature inputs from sensors both in the hypothalamus itself, in the skin, vessels etc. The "after-drop" in connection with rewarming of hypothermic patients first described in the Dachau experiments is due to the stimulation of cutaneous sensors which leads to a redistribution of blood to the peripheral tissues (Alexander 1945, Burton and Edholm 1955). This "afterdrop" is thought to be responsible for the death of 16 people who fell in the water of Greenland, rescued after short time and wrapped into warm covers (Marcus 1979). However, this "after drop" effect is also found to take place during heat-exposures at normal body temperature and has probably the same explanation as the "afterdrop" found to take place in hypothermic patients, namely stimulation of the cutaneous "thermo-receptors" (Opstad et al 1991). The seriousness of hypothermia is dependent upon the combination of the exposure time, and the degree of hypothermia. Ventricular fibrillation or asystole is by some researchers thought to be due to a temperature difference between the endocardium and myocardium of more than 0.5 to 1.0 °C. This causes the difficulties with blood rewarming of hypothermic patients, which should therefore be performed carefully by external rewarming of the heart or under thorough cardiologic surveillance or by the aid of a heart-lung machine (Lloyd 1986).

The thermoneutral environmental temperature for humans is between 28 and 30 °C. In our climate we are therefore dependent on cloths, houses or other type of shelters to protect us against the cold and the development of hypothermia. During the ranger training course all subjects wear the same type of uniform, carry the same weight (rucksack) and have roughly the same physical activities. This may sometimes cause temperature regulation problems due to individual differences mainly in heat production, subcutaneous fat and physical fitness.

During the ranger course we have shown that there is a reduced hypothalamic set-point temperature, since core temperature was reduced in spite of increased skin temperatures. Even during a bicycle exercise test the core temperature was lower than in the control experiment, in spite of increased skin temperature and increased spill-over of heat during work (Bahr et al 1991, Opstad and Bahr 1991). During the course the cadets may therefore be more exposed to hypothermia but more protected against frostbite than in a control situation. Clinically we have seen several cases of hypothermia with temperatures as low as 32.4 °C. The cadets were still conscious, and able to walk, but they were not cooperative and were mentally changed and unreliable. One cadet got a small injury to his knee during an obstacle race and was therefore immobilized. He became unconscious within 30 minutes due to hypothermia. However, he recovered relatively quickly when he was brought indoors at a room temperature of about 20 °C and wrapped into blankets.

As shown in Figure 1, core temperature may vary between 36 and 39 °C mainly due to variations in exercise levels during the course. The cadets may also be exposed to high environmental temperature during the course, and we have had several cases of a moderate heat stroke. At high environmental temperatures the physical exercise is performed at night time, and the cadets are given extra salt and liquid. The beneficial effects of food for cold tolerance are well established (Åstrand and Rodahl 1986). The interaction between cold tolerance and sleep deprivation is hardly investigated. There are, however, indications that cold tolerance is impaired during sleep deprivation (LeBlanc 1987).

Also heat stress reduces the subjects' exercise capacity, although there are some adaptation to hot climate through increased sweating capacity that can delay exhaustion (Åstrand and Rodahl 1986, Opstad et al 1991, Sawka et al 1993, Nielsen 1994, Febbraio et al 1994). When the temperature exceeds 30 ° C most physical activities are stopped or the intensity is reduced. If activities have to continue in spite of high environmental temperature, it is extremely important to have a sufficient and regular intake of water and salts, since drinking based on the cadets' thirst will replace only 2/3 of the liquid needed, and only a moderate dehydration will lead to dramatic impairment of physical performance (for review, see Armstrong et al 1993, Francesconi et al 1993, Gisolfi 1993, Keen 1993, Armstrong 1994, Fortney and Miescher 1994, Greenleaf 1994, Hubbard et al 1994, Ivy 1994, Knochel 1994, Lamb 1994, Nose et al 1994a, b, Rolls 1994, Sawka and Neufer 1994).

1.6 Consequences of sleep deprivation

The exact role of sleep is unknown. However, the physiological and psychological consequences of sleep deprivation are well established. First, sleep deprivation affects subjective well-being, ability for social care and mood. Further, mental performance, evaluated with psychometric performance tests, is affected. As sleep deprivation proceeds, periods of microsleep or lapses appear more and more frequently and for prolonged periods of time with strongly reduced attention. In work paced tasks, this leads to an increased amount of omissions. In contrast, there is no marked increase in the number of wrong responses. The reaction speed is also reduced so each task needs more time to be fulfilled properly. Complex tasks may finally be impossible to perform because the subjects are not able to cope with many tasks or large amounts of information simultaneously. The capacity to learn is reduced, and the performance of new tasks are particularly sensitive to the effects of sleep deprivation. Therefore overlearned tasks are far more resistant to the effect of sleep deprivation than new and untrained tasks. This is a main argument for drilling certain critical skills, that the soldiers are dependent on when they are sleep deprived, physically exhausted and in a survival situation (Williams et al 1966, Passnau et al 1968, Ainsworth and Bishop 1971, Johnson and Naitoh 1974, Horn 1978, Opstad et al 1978, Bugge et al 1979, Nicholson and Stone 1982, Haslam 1983, 1984, Angus et al 1987, Bonnet 1987, Gaillard 1987, Jouvet 1987, Opstad 1987a, Rousel 1987, Shapiro and Driver

1987, Borbély and Tobler 1989, Linde and Bergstrøm 1992). Endurance capacity is strongly affected by sleep deprivation (Williams et al 1965), particularly if the task is monotonous and boring. This is the reason why driving vehicles is so sensitive to the effects of sleep deprivation. In contrast, if the test time is short, and the cadets are activated just before the test, the subjects can perform normally for a prolonged period with sleep deprivation. When sleep deprivation extends beyond 3 days more serious neurological dysfunction will appear. These are balance and coordination disturbances, nystagmus, visual hallucination and disorientation for time and site (Ross 1965, Wilkinson 1965, Kollar et al 1966, 1968, Wilkinson et al 1966, Pasnau et al 1968, Haggard 1970, Sassin et al 1970).

There is also alteration in the subjects' perceptual defence, measured with a Defence Mechanism Test (DMA), during the ranger training course, which indicates a massive increase in integrative mechanisms, whereas the self-assertive tendency remained unchanged (Myhrer 1987). It has been speculated wheather this alteration may be related to the decreased testosterone levels which well may promote such mental changes. Reality is probably more complex, and a main reason for alterations in mental function during the course is sleep deprivation, which does not have any major role in the decreased testosterone secretion during the course. Therefore the decrease in androgens is probably only one factor contributing to this type of changed mental function.

Rather moderate endocrine alterations have been demonstrated during sleep deprivation (Palmblad et al 1979, Horn 1992, 1993). The decreased levels of unconjugated adrenal androgens during sleep deprivation were largely attributed to the effect of decreased physical activation (Åkerstedt et al 1980). It has been speculated wheather there might be metabolic substances whose secretion is stimulated by sleep deprivation or physical fatigue, or that even "metabolic break down products" might have a role in the induction of sleep or wakefulness. Many rather simple theories have been proposed, such as the monoaminergic explanation put forward by Jouvet (1969, 1972) with the connection between the raphe nuchlei (serotonin) and Slow Wave Sleep (SWS) and the locus coeruleus (noradrenaline) and Rapide Eye Movement Sleep (REM sleep). Many investigators have tried to give a molecular explanation of the restorative effects of sleep (Adam 1980, Oswald 1980, Walker and Berger 1980, Crick and Mitchison 1983, Krueger and Obal 1993 a, b), and it is shown that glycogen content of the CNS is restored during sleep (for review see Benington and Heller 1995). Still the concept is valid that many of the basal rhythms such as spindles (7 to 14 Hz), the alpha rhythm (10 Hz) and the slower cycles with 1 to 4 Hz (delta rhythm) are endogenous for the thalamus but are modulated by impulses from different parts of the brain such as nucleus suprachiasmaticus, locus coeruleus, the raphe nuclei, hypothalamus, etc. and even from the cortex itself (Steriade et al 1990, 1993, 1994, Jones 1993). Recurrent slow waves (less than 1 Hz) generated in the cortex may also modulate the thalamic rhythms (Steriade 1994). The excitatory and inhibitory amino acids glutamate and gamma-amino butyric acid are the main neurotransmitters by which the neocortex and the thalamus process synaptic information.

The more slowly acting neurotransmitters such as acetylcholine, noradrenaline, serotonin and histamine are thought to control some of the input to the thalamus, thereby modulating the state of activity of the thalamo-cortical neurones (Tsumoto 1990, Crunelli and Leresche 1991, Llinas and Pare 1991, McCormick 1992a, b, Halasz 1993).

During the last decades, many sleep-inducing substances have been described both in the CNS, the cerebrospinal fluid, and in the peripheral circulation, e.g. the delta sleep inducing peptide (Monnier and Hösli 1964, Monnier et al 1975, 1977, Pappenheimer et al 1975, Pappenheimer 1982, Jouvet 1987, Borbély and Tobler 1989, Charnay et al 1990, Inoue et al 1990, Vallet et al 1990, Bjartell et al 1991, Friedman et al 1994). However, many of these sleep-promoting substances have other primary functions. This is the case for cytokines, tumor necrosis factor, interleukins, interferons, muramy peptides produced by macrophages, bacterial peptoglycan and prostaglandins (Johannsen et al 1990, 1991, 1994, Krueger and Majde 1990, Krueger et al 1990b, Toth and Krueger 1990, Kapas et al 1991, 1992, 1993, Kapas and Krueger 1992, Obal et al 1992 a, b, Payne and Krueger 1992, Toth et al 1992, 1994, Krueger and Obal 1993a, b, Payne et al 1993, Kimura et al 1994, Krueger and Toth 1994, Krueger and Majde 1995). However, none of these cytokines or TNF changed during the course to any significant amount, except for cytokine 6 which decreased slightly (Bøyum et al 1992). It is an old clinical experience that infections are accompanied by increased amount of sleep, this has been particularly well documented after inoculation with the influenza virus (Krueger 1990, Krueger and Majde 1990, Kimura et al 1992).

Intracerebroventricular administration of vasoactive intestinal peptide (VIP), or the structurally homologous pituitary adenylate cyclase-activating peptide, is shown to enhance REM sleep (Bredow et al 1994, Obal et al 1994). The plasma levels of VIP increase during the course (Øktedalen et al 1983b, Opstad 1987c). An indication that even other unknown metabolic substances may induce sleep is that hemodialysis is shown to ameliorate the subjects' well being, state of mood and physical fitness, independent of peptides known to induce sleep, such as delta sleep inducing peptide, corticotropin releasing hormone, alpha melanocyte stimulating hormone, met-enkephalin, beta-endorphin, beta-lipotropin and beta-endorphin (Hegbrant et al 1992). It is, however, unclear if there is any significant secretion of these substances during the course. Urine has been collected during the courses, and several peptides have been extracted. Some of these peptides might induce sleep (Trygstad et al 1980).

By use of adrenergic agonists, particularly those which easily cross the blood brain barrier, such as amphetamine, it is possible to extend performance for some time. However, these substances cannot replace sleep, and since they do not produce "new energy" but only squeeze already existing "energy reserves", the use of these substances will aggravate exhaustion and prolong the subjects' recovery period (for review see Opstad 1993, Cochran et al 1994). Also coffee is well known to enhance both mental and physical performance (Nehlig et al 1992, Penetar et al 1993). Unfortunately caffeine does not provide new

energy, but aggravates exhaustion and prolongs the recovery period. During the last year a new drug has been described, modafinil, which stimulates wakefulness without the negative side effects of amphetamine-like drugs, such as the increased rebound effects. Modafinil is mainly an alpha-1 agonist and acts postsynaptically to enhance wakefulness. This drug has become popular in the treatment of narkolepsia, particularly in France where the drug was discovered (Duteil et al 1990, Jouvet et al 1991, Lyons and French 1991, Lin et al 1992, Bourdon et al 1994). However, there has been a dispute on the minimum amount of sleep necessary to be efficient as recovery sleep (Meddis et al 1973, Naitoh 1981, Naitoh et al 1982, Angus et al 1987, Bonnet 1987a, b, Åkerstedt et al 1993).

The significance of very short periods of sleep, less than 5 minutes, was demonstrated during a Guinness record attempt for marathon swing dance some years ago (Opstad unpublished). The dancer experienced hallucinations, illusions and misperceptions at approximately 55-60 hours of sleep deprivation, and at 65 hours he saw holes in the floor and left the dancing room because he thought that the other participants were homosexuals. Three years later the same dancer wanted once more to attack the Guinness record of 97 hours in marathon swing dance. This time the group trained physically for 6 months before the attempt. The training consisted mainly of endurance training and dancing, in order to be able to physically stand the strain. The subjects were carefully taught beforehand of all symptoms of sleep deprivation and of other problems they could expect to be faced with. The rules allowed 5 minutes of rest for each hour of dancing. These five minutes should bee used for visit to the toilet, eating, drinking and maintenance of equipment. A quiet room was prepared next to the dancing theatre, containing their own private bedding which they associated with sleeping. In the dancing theatre the circadian rhythm was maintained by alterations in lightening in accordance with outdoor light. The 5 minutes periods during day time were used for all practical purposes such as eating, drinking and maintenance of the equipment. The 5 minutes periods at night were used for sleep. Some minutes before the sleep periods, the light intensity in the room was reduced, and the music was dampened, monotonized with the same melodies in the same order. This system was administered by helpers so the participants should not be concerned with that part. By this methods the Guinness record was increased from 97 hours to 110 hours of marathon swing dance without any serious mental or physical disturbances during the whole period. The experiment was stopped at 110 hours because it was New Years Eve, the record had been made, and the dance institute and Guinness' representatives were not prepared for any longer attempt. This experiment shows that even very short sleep periods are efficient to sustain mental performance during continuous operations. Obviously all sleep that has a longer duration will be even more beneficial.

There are large variation in sleeping time among species, even between those who are physiologically comparable such as the shrew and bat, and between vegetarians and meat carnivores (Meddis 1975). The issue of whether sleep is physiologically necessary has been unresolved until the series of experiments in rats by Rechtschaffen and his colleagues (Rechtschaffen et al 1983, 1989a, Mistlberger et al 1987, Benca et al 1989, Bergman et al

1989a, b, Everson et al 1989a), showing that all rats died or were sacrificed when death seemed imminent within 11 to 32 days after total sleep deprivation (Eversen et al 1989b), within 16 to 54 days of paradoxical sleep (REM) deprivation (Kushida et al 1989a) and within 23 to 66 days of slow wave sleep or non rapid eye movement (NREM) sleep deprivation (Gilliland et al 1989). The sleep deprivation syndrome that included death was preceded by debilitating appearance, skin lesions, increased food intake, weight loss, increased energy expenditure, decreased body temperature, increased plasma noradrenaline and decreased plasma thyroxin (Kushida et al 1989b, Rechtschaffen 1989b). Three out of eight rats with the strongest temperature decline died even after two to six days with recovery sleep. There are no such results available from other species, and it might be difficult to draw absolute conclusions as to the limits of tolerance for humans. First of all it is difficult to totally sleep deprive humans for longer periods of time, and this period should probably not exceed 10 days. During the Norwegian ranger training the cadets get 2-4 hours of sleep between activities during a 5-6 day course, and if the course is prolonged, extra sleep is provided. However, due to rather large interindividual variations in the cadets' tolerance to sleep deprivation, a close clinical surveillance will be important to prevent harmful effects.

1.7 Circadian rhythm

Most biological parameters show infradian, circadian and ultradian rhythms and even rhythms lasting for only a few minutes (Aschoff 1979, Krieger 1979, Webb 1982, Hallberg et al 1983, Touitou and Haus 1992a, b). The most pronounced rhythm in humans is the circadian rhythm which is regulated from the nucleus suprachiasmaticus in the hypothalamus. Mental circadian rhythm as well as deep body temperature increase to a maximal level during the afternoon and to a minimum level during the night time. There is information that this circadian decrease in deep body temperature, and thus probably also mental performance, is regulated by melatonin. Melatonin is secreted in the pineal body and influences the nucleus suprachiasmaticus as well as other brain regions. The melatonin secretion and also its effects on target tissue are reduced with increasing age. This might partly explain the reduced tolerance to changes in the circadian rhythm in aged people compared to young ones. The most efficient way to adapt subjects to a new circadian rhythm is to expose them to bright light or to give them melatonin (Åkerstedt et al 1979, 1982, Czeisler et al 1980, 1992, Cagnacci et al 1995, Dawson et al 1995, Honma et al 1995, Touitou 1995).

In contrast to the circadian rhythm for mental performance, steroid hormones decrease during day time and increase during night time. For catecholamines circadian rhythms have been demonstrated for urine excretion and are probably a reflection of day activity and night rest. Thyroid hormones have a long half-life in plasma and therefore show very small circadian variations in contrast to thyroid stimulating hormone which increases during the afternoon to a maximum level at midnight (for review see Krieger 1979, van Cauter and

Turek 1995, Paper VII). All effects of sleep deprivation are more pronounced at night than during the day, even so for the serious neurological symptoms of sleep deprivation. Hallucinations are very rare during daytime.

1.8 Stress, training and overtraining

Overtraining is defined as a reduction in performance in spite of normal or increased training intensities and in the absence of disease. The reason for overtraining is an imbalance between training and rest, very often because the training intensities and frequencies increase too fast (Israel 1958, Kindermann 1986, Kupiers and Keizer 1988, Kibler et al 1992, Fry et al 1992, 1994a, b, Perry 1992, Puffer and McShane 1992, Bahr et al 1993, Budgett 1994, Hart 1994, Morgan 1994).

Regular training is the stimulus necessary to increase or maintain a high muscle strength and physical performance. During training the body is in a catabolic state which means that energy production and performance is dominating over tissue repair and anabolism. This catabolic phase is regulated by the autonomic nervous system and the so called catabolic hormones which are the catecholamines, glucocorticoids, thyroid hormones, glucagon and some peptides such as vasoactive intestinal peptide (VIP). In contrast, tissue proteins and function, muscle size and strength etc., increase during the anabolic phase, which most often takes place during recovery or rest. This anabolic phase is stimulated by anabolic hormones such as testicular and adrenal androgens, growth hormone and insulin, which have a circadian rhythm with a strong increase during night time. This increase is also stimulated by sleep and food. To profit maximally of the training it is also important to conduct a regular life with frequent and long enough periods of rest and not least take into account interactions with the circadian rhythm and sleep. Prolonged periods of military field training with limited amounts of sleep and food represent an extreme catabolic state with high priority to energy production and consequently low priority to anabolic metabolism. During the ranger training course a decrease was found for the cadets' mechanical efficiency from 24.6 % normally to 20.9 % after 4-5 days of continuous stress. The mechanisms of these alterations may be similar to the state of overtraining in elite athletes. Overtraining has been defined as incomplete recovery from exercise, causing a decline in performance capacity. The hormone and metabolic profiles of overtrained athletes are similar to those found during the ranger training course (Kuipers & Keizer 1988, Lehmann et al 1991, 1992a, b, c, 1993, Fry et al 1992, 1994a, b, Parry-Billings 1992, Hooper et al 1993, Roberts et al 1993, Martinelli et al 1994).

The "soft tissue overuse syndrome" is present in one or another form in most of the cadets during the course, most common in the lower extremities, such as the ileo-tibial band friction syndrome, patellar tendinitis, Achilles tendinitis, plantar faciitis, or stiffness of the groin area due to tendinitis of the adductor/tendon units, particularly the adductor longus. The cadets starting the course with some sort of tendinitis in the lower extremities very

often have to be withdrawn from the course. Those who are poorly trained before the course (particularly march training), with new boots, often develop soft tissue overuse syndrome early in the course. Most of these cadets are able to complete the course with some adjustment of the amount and type of exercise, physical support for the injured joint, and if necessary paracetamol as analgesic and antiinflammatory drug. Nonsteroidal anti-inflammatory drugs should be used very carefully due to the gastrointestinal alterations during the course (Øktedalen et al 1983, 1992). Steroid injection in the injured area is not practiced since it has been shown to weaken the tendon and might lead to ruptures (Stannard and Bucknell 1993). With this treatment most of the cadets are able to fulfil the course, and their symptoms often ameliorate during the course in spite of ongoing physical activities. This is well in accordance with several investigations from sports and occupational medicine, showing that a certain level of adapted activity and not absolute rest, in addition to regular stretching, is advantageous for the healing of soft tissue overuse syndromes (Fyfe and Stanish 1992, Galloway et al 1992, van-Mechelen 1992, Giffin and Stanish 1993, Jones et al 1993, Almekinders and Almekinders 1994, Frontera et al 1994, Hart 1994, Karlsson et al 1994, Kvist 1994, Mascaro and Swanson 1994, Torstensen et al 1994).

It is also documented that the healing of the injured tissue is stimulated by training of other muscle groups. This might indicate that training elicits general responses which have effects beyond the trained muscle group. It is obvious that the healing of soft tissue overuse syndrome is influenced by the general endocrine and metabolic state of the body which during the course is in an extreme catabolic state. However, the increased levels of glucocorticoids and catecholamines are expected to dampen the inflammatory process but probably also the healing effect. In addition there is no significant increase in interleukins during the course (Bøyum et al 1992), which would stimulate the inflammatory process. It might also be that the "extra rest" provided for the injured cadets may be enough to ameliorate the cadets' general metabolic state and favour healing of the injured areas. There is unfortunately no information available on this topic today.

1.9 Trench foot, skin blisters and ulcers

Skin blisters, gall, erosions and/or ulcerations are common during military training courses, and almost all cadets have experienced this problem more or less. One course had to be stopped after 3 days because only 3 or 4 cadets out of approximately 30 had feet able to continue the training program. When infections appear with cellulitis and lymphangitis the cadets have to be withdrawn from the course.

There are large inter-individual variations in the time and conditions needed to develop these problems. This might be due to differences in epidermal cell cohesion, cell tolerance to pressure and stretching, foot sweating, the anatomical shape of the foot and the footgear. Dry or very wet skin surface produces less friction and thereby less blisters than moist skin,

which increases the shearing forces. Friction blisters are not second degree thermal burns, however, blisters form faster as the skin temperature increases, and clinically it is observed that blisters occur more readily in warm than in cold environment (Naylor 1955a, b, Cortese et al 1969, Knapik et al 1992). If these above mentioned problems are associated with low environmental temperatures for a certain period of time, the real trench foot may develop with irreversible damage to the foot as a consequence (Czerniecki and Ingaramo 1987, Fritz and Perrin 1989, Haller 1990, Parsons et al 1993).

Friction blister develops only if the stratum corneum is sufficiently thick and resistant to withstand the frictional forces transmitted into the epidermis. If the stratum corneum is thin, an erosion will be the result. Blister-prone skin is relatively immobile and bound to deeper tissue. The differential movements between the layers of the skin cause the shearing forces that result in a cleft into which fluid passes. Therefore blisters most easily are localized to the soles, palms and tops of the toes, whereas abrasions are localized to the groin and axillae.

Blisters may develop within hours of friction and are almost always localized to the epidermis in the middle and upper Malpighian layer. The roof is composed of the stratum corneum and stratum granulosum with both normal and damaged prickle cells. The base consists of normal basal and prickle cells (Cortese et al 1968a, b). Some cells may be eosinophilic, may demonstrate vacuolization and nuclear pycnosis, and they may be elongated as if they where mechanically stretched. The dermis shows small variations, and blood vessels may be slightly dilated and congested. Dermal sweat ducts are preserved. Unlike other blisters there is no invasion of blood cells. Within some hours the cleft will be filled with fluids due to the hydrodynamic pressure. If the affected hand is elevated over the head the blister will not be filled with fluids (Sulzberger et al 1966). The blister fluid reflects the composition of serum, however, with slightly lower protein concentration than serum. It does not clot and contains no platelets, fibrinogen or fibrinolysin (Cortese et al 1968b, Akers and Sulzberger 1972, Akers 1977, 1985, Comaish 1973, Levine 1982).

If the blister fluid is evacuated 2-3 times within 24-48 hours this may result in blister roof adherence to the base, which will reduce the discomfort, protect the base from dehydration, maceration, infections and the formation of a thick crust, which would act as a barrier to epithelial migration, and retard healing (Cortese et al 1968a, Harris 1979). If the blister roof is torn off, the blister base should be covered with artificial skin, which, however, does not have the same qualities as the natural skin, or with ointment on a thin gauze pad, which should be taped to protect the blister base against further erosions and prevent it from dehydration. Fenestration in the blister top to allow a continuous release of the fluid (Stokes 1965) is, however, not recommended due to the above mentioned reasons.

Good hygiene, with if necessary the use of antiseptic solutions, may be extremely important to prevent infection. An antibiotic ointment is used to treat infected blisters but may also be used as a prophylacticum. If the infection proceeds to cellulitis, and

lymphangitis, systemic antibiotic treatment may be necessary as well as withdrawal from the course.

To prevent friction blisters, erosions or galls, well-fitted footgear is important, two or more socks which are smooth on the side turning to each other. This places the point of maximal frictional shearing force between the socks. The socks should be made of a material which absorbs moisture and by that keeps the feet drier (Herring and Richie 1990, 1993). Different types of powder may also reduce the frictional shearing force and also absorb moisture. The powder is, however, not comfortable if the footgear become wet. Salicylic tallow makes the skin soft, prevents the skin from absorbing water and reduces the friction shearing forces. Application of antiperspirants to the feet may also reduce serious foot injuries (Darrigrand et al 1992). However, to avoid moisture it is important to have time in-between activities to change or dry the socks and footgear and to apply salicylic tallow. March training and good hygiene before the course may also be important to prevent galls and blisters during military training.

The generation of blisters, galls, erosions and trenchfoot may be aggravated by the decreased peripheral nerve sensitivity that frequently appears during prolonged physical strain because the cadets may not recognize the problem at a stage when it is time to prevent more serious damages. Most cadets have a decreased sensitivity in their feet during the course, most often in one or several toes or in the foot sole. The paresthesia, numbness or decreased sensitivity often take several weeks or even months after the course to recover completely. Similar sensitivity losses may take place during sports activities or other type of exercise (Goetz et al 1994), vibration (Bismar and Ekenvall 1992), disturbance of the venous circulation (Shami et al 1993), continuous physical exercise (Pistorius et al 1994) and starvation, whereas the role of sleep deprivation is unknown. The development of neuropathy is presumed to be worsened by cold exposure (Chen et al 1994) while regional hypothermia may protect the nerve from tourniquet neuropathy (Kelly et al 1992).

1.10 Health risk and the development of diseases

Selye introduced the concept of stress and that stress could lead to disease (Selye 1936, 1946, 1950, 1970, 1978, Kagan and Levi 1971, Glavin 1991). This was elaborated in his theories on the general adaptation syndrome and also in his many studies on gastrointestinal diseases, particularly the gastrointestinal ulceration. During the ranger training courses of the Norwegian Military Academy, many of the same gastrointestinal alterations have been observed in healthy young men without previous history of gastrointestinal disease (Øktedalen et al. 1982a, b, 1983a, b, c, d, e, f, g, 1984 a, b, 1988). The main finding was 3-4 times increase in both gastric acid concentration and production. In addition a 5-10 fold increase in bile acids was produced. The mucosal microcirculation is normally higher in the curvatura major than in the curvatura minor. This was reversed during the course, and consequently we found a rather dramatic decrease in the blood

circulation in the curvatura major. This place, the prepyloric part of the curvatura major, is also the localization for most of the mucosal erosions found in most of the cadets who were subjected to gastroscopy (Nesland et al 1989, Øktedalen et al 1992). The fact that gastrointestinal symptoms such as dyspepsia, abdominal pain, vomiting etc. appear early in the course, often during the first few days, and are not really aggravated during the course, might indicate a strong psychological component. In the beginning of the course the cadets know that they have almost a week of extreme stress ahead. This might induce aversion against (psychological defence) the whole training program, the course and the officers. The symptoms may also depend on the type of leadership since we have seen rather large variations in gastrointestinal problems from one course to the other. Another explanation may be the high ketone levels in blood found on days 2-3 during the course.

Fortunately, with rather simple measures we can avoid most of the gastrointestinal problems, first of all by regular drinking of water or solutions that neutralize the surplus acid production. Small meals, in the form of bread and milk or tea, or also in the form of soup, may dramatically reduce the cadets' gastrointestinal problems. If this is not sufficient, the surplus acid secretion and the following gastrointestinal problems can be avoided by a H2 antagonist (Cimetidin) and probably also by other pharmacological agents that reduce the gastric acid production (Øktedalen et al 1983d). The diarrhea often seen in marathon runners is not observed during the ranger military training course. Due to the reduced intake of food, the cadets have only one defecation during the course, and this is usually of normal constituents.

Gastrointestinal regulatory peptides such as gastrin, secretin, pancreatic polypeptide, vasoactive intestinal peptide (VIP), and gastric inhibitory peptide all increase during the course. VIP and secretin normalize after a meal or glucose ingestion. The VIP response to a bicycle exercise test is increased during the course but abolished by simultaneous glucose infusion (Øktedalen et al 1983b, c, Wiik et al 1985, 1988, Opstad 1987b).

There is no sign of hepatic malfunction during the course, hepatic enzymes do not show major changes, whereas a moderate increase may appear in myocardial enzymes. Atrial natriuretic peptide (ANP) in plasma increases in patients with various heart diseases such as congestive heart failure. During the ranger course there was no major increase in ANP levels, and the ANP response to a bicycle exercise test was rather decreased than increased. No significant alterations were found for plasma electrolytes except for serum chloride which decreased during the course. During the bicycle exercise test the serum chloride increase was abolished by glucose infusion (Opstad et al 1994). These results suggest that the cadets are not exposed to any cardiac work overload during the course.

1.11 Immune function and stress

During the last years a number of pathological consequences of stress have been demonstrated, such as reduced immune function, increased risk for cancer development,

faster ageing and activation of many diseases (Morley et al 1991, Sternberg et al 1991, Verrier and Carr 1991, Yirmiya et al 1991, Falek 1993, Irwin 1993, Stein and Miller 1993, Ursin and Olff 1993, Ursin 1994, Baum et al 1994, Cacioppo 1994, Eliot 1994, Glaser and Kiecolt-Glaser 1994, Pedersen et al 1994, Shephard et al 1994, Nilssen et al 1995).

During the Norwegian military training course alterations have been found for immune related parameters in blood (Bøyum et al 1992). However, there has not been any clinically evident tendency for infections during or after the course (Opstad unpublished).

During the course there is an increase in the number of neutrophil granulocytes and monocytes, and a reduction of eosinophils and lymphocytes, and small alterations for the basophil granulocytes. This should indicate a certain activation of the cellular, non-specific immune function. The decrease in the number of lymphocytes could indicate a certain inhibition of specific immune function, but may nevertheless simply reflect a changed distribution of lymphocytes within the body. Also all the subgroups of lymphocytes (CD4 T cells, CD8 T cells, B cells and NK cells), decreased in the same way. However, no significant alterations were found for interleukin 1, 2, and 4 during the course, whereas a decrease was found for interleukin 6 at the end of the course. A decrease was found for all immunglobulins, most pronounced for IgM which decreased by 20-35 %, IgA by 10-20 % and IgG by 10-15 % (Bøyum et al 1992, 1995). No significant alteration was found for TNF measured in plasma by radio-immuno-assay, whereas a slight increase was found when measured by a bio-assay. In addition an increased TNF response was found after stimulation of monocytes (Løvhaug personal communication).

Most of the cadets who have been taken out of the course due to infections, suffered from viral or bacterial diseases already at the start of the course. Cadets with fever combined with suddenly reduced physical performance, exercise induced dyspnoe, or headache, just before or in the beginning of the course, were automatically taken out of the course to prevent serious complications, including sudden death due to viral myocarditis (Norton 1990, Camerini et al 1991, Drory et al 1991, Shepard and Shek 1994). Cadets with other cardiomyopathies, such as severe arteriosclerosis, hypertrophic cardiomyopathies, coronary artery anomalies and arythmogenic right ventricle, would not have been accepted as student at the academy since such conditions have been shown to represent a great risk for sudden cardiac death in combination with strenuous exercise (Hawley et al 1990, Burke et al 1991).

In an investigation of the American ranger training course an increased frequency of clinical infections was demonstrated particularly in the form of cellulitis of the lower extremities and particularly at the final part of the course. This alteration was followed by similar alterations as those described by Bøyum et al (1992), with decreased plasma concentrations of cytokines, particularly cytokine 6 (Moore et al 1992, Beisel 1994, Shippee et al 1994). A decreased antibody response to two standard vaccines was also

found, as well as decreased lymphocyte responses to mitogens, diminished delayed dermal hypersensitivity responses and decreased NK cell activity.

A certain type of asthma may be exercise-induced (Brusasco and Crimi 1994, Hough and Dec 1994, Kobayashi et al 1994, Makker and Holgate 1994), however, no asthma or allergic reaction has been observed during the ranger courses. Cadets who start the course with allergic airways get better during the course. This may be explained by the high levels of glucocorticoids and catecholamines during the course.

1.12 Haematological alterations and stress

A 15-25 % decrease was found for Hb/Ht during the course. This decrease was followed by a decrease in haptoglobin, increased ferritin, decreased serum iron and total iron binding capacity (TIBC) and increased bilirubin. An increase was also found for the plasma erythropoietin activity (Lindemann et al 1978) and for the number of reticulocytes (Bøyum et al 1995). Others have found a decrease in erythropoietin concentration during a similar course, which probably is due to dehydration which is known to stimulate erythropoietin secretion (Gunga et al 1995). The endocrine alteration during the course, such as reduced thyroid activity, is shown to affect erythropoietin concentration in plasma and by that also erythropoiesis (Blanchard et al 1993, Fadry et al 1994).

No significant alterations were found for osmotic fragility, whereas the mechanical fragility of the erythrocytes decreased continuously during the course. All these alterations were mainly due to the effect of physical exercise, whereas sleep and food deprivation did not play any major role (Opstad et al unpublished). All these alterations indicate hemolysis as the most plausible explanation for the decreased hemoglobin and hematocrit during the course. This is also supported by a decrease in the plasma levels of haptoglobin and an increase in plasma ferritin levels during the course (Vidnes and Opstad 1981, Moore et al 1993). Among the hematopoietic growth factors measured, interleukin 3 and granulocyte CSF did not change, whereas a significant increase was found for granulocyte-macrophage colony stimulating factor (Bøyum et al 1995).

Rather large interindividual variations in Hb/Ht have been observed. Some cadets have as low Hb/Ht as 50 % of their precourse levels. A decrease of 15 - 25 % in Hb/Ht should represent 1.5 liter of full blood which normally should give a rather dramatic decrease in physical and mental performance particularly for endurance performance. To our astonishment this was not the case, even those with very low hemoglobin concentrations are relatively unaffected with regard to physical and mental performance. One possible explanation for this is that the mechanically weakest erythrocytes are also those with the poorest oxygen transport capacity and vitality, and consequently oxygen transport capacity is less affected than the corresponding Hb/Ht. These alterations in Hb could not be explained by alterations in plasma volume, since only small alterations were found for plasma total proteins or albumin during the course, and small variations were found for

plasma volume measured by an isotope method (^{125}I-albumin) (Lindemann et al 1978, Opstad et al unpublished).

1.13 Serum enzymes and lipids during stress

Rather large increase was seen in all muscle enzymes during the course. This increase is due to the effect of physical exercise and is not influenced by energy or sleep deficiency (Opstad unpublished). In contrast the increased levels of urea, uric acid, creatinin and the decreased levels of glucose were mainly due to energy deficiency. In clinical medicine increased plasma levels and CK (creatine kinase), LD (lactate dehydrogenase), ASAT (aspartate aminotransaminase) and ALAT (alanine aminotransaminase) are normally considered to reflect tissue damage or necrosis and are important diagnostic tools for many diseases such as myocardial infarction (Zimmerman and Henry 1974). During the ranger training course isoenzyme analysis has shown that almost all of the increase in plasma enzymes during the course originates from striated muscles. It is now well established that these enzymes also increase during sports competition as well as during ordinary physical training (Meltzer and Moline 1970, Critz et al 1972, Loegering et al 1975, Friedel et al 1976, Armstrong et al 1983, Armstrong 1984, Purroy-Unanua and Gonzales-Buitrago 1985, Song 1990, Smith et al 1992). Since physical training, and most sports competitions, normally lead to increased muscle mass and strength, the increased plasma enzyme levels during the course can not be explained by tissue damage or necrosis or even overtraining, but should rather be taken as an expression of the increased energy turnover during physical exercise and that these enzymes have a limited lifetime which is reduced by increased energy turnover. It is established that untrained have a larger increase in these muscle enzymes than trained subjects (Noakes and Carter 1982).

Serum cholesterol, triglycerids and lipoproteins, such as Apo B, Apo A-I and Apo A-II, decrease during the course due to the physical exercise and are unaffected by food or extra sleep (Magnus et al 1984, Magnus et al unpublished, Opstad unpublished). In contrast, an increase was found for free fatty acids (FFA), β-OH butyrate or glycerol. After a bicycle exercise test the FFA levels in serum reached almost toxic levels (Rognum et al 1981).

1.14 Aims of the present study

The present investigation focuses particularly on the endocrine and metabolic alterations during prolonged physical strain day and night, and we have also attempted to sort out endocrine and metabolic changes caused by food and sleep deficiency (Paper 1).

Possible mechanisms for physical and mental exhaustion as well as the role of conjugated catecholamines have been studied in three separate papers (Papers 2, 3, 4).

Another contributing factor to exhaustion, reduced performance or impaired recovery may be decreased androgen secretion. The relationship between the alterations in adrenal and testicular androgens and the regulation of their secretion have been investigated in three separate papers (Papers 5, 6, 7).

Alterations in the circadian rhythm of the different hormones and their relation to mental performance have been investigated in Paper 7.

2 METHODOLOGICAL CONSIDERATIONS

2.1 The experimental model

All experiments were performed in male cadets from the Norwegian Military Academy during their ranger training courses at the end of the first year (June) or beginning of the second year (August-September) at the Academy. The course is part of the cadets' obligatory training program at the Academy. In spite of this we were allowed to do some standardization of the training program and to introduce differences between groups in order to study for example the impact of different stress factors. The cadets were between 21 and 28 years of age and physically well trained and healthy. Because it was difficult to predict who of the cadets would be accessible at the time of the planned investigation during the course, we often preferred to do the control experiments 2-3 month after the course. By this procedure we did not have to perform control experiments in subjects who did not complete the course. Experiments which have been performed both before and 2-3 months after the course have shown that full endocrine recovery is obtained 2-3 months after the course.

Although the Norwegian Military Academy also has 1-3 female cadets each year, they were not included in the research program due to the hormonal differences between the two sexes and differences due to the female regular periods.

The courses normally lasted from 5 to 7 days. They started on a Monday morning or Sunday afternoon and finished on the following Friday night or Saturday morning. The cadets had continuous physical infantry activities around the clock corresponding to 35 % of their maximal oxygen uptake (Waldum and Huser 1974, Aakvaag et al 1978a) as measured by continuous heart rate recordings. The activities consisted of patrols during night time and during the day; combat training with attacks, building of defence positions, passing obstacles and even through narrow tubes containing water.

The work load corresponded to a daily energy consumption of 34.000 to 46.000 KJ, which is in accordance with the estimated energy loss during the course. The total weight loss depended on the food supply during the course. In the middle of the course two cadets co-

operated to slaughter a hen, boil it on their primus and consume it. This half hen contains mainly proteins which represent an energy content of approximately 2500 to 3400 KJ for each cadet and is often the only food they received. For these cadets the weight loss reached 8-12 kg during the course. However, in some courses, the cadets consumed as much as 4200-6300 KJ daily in the form of bread, biscuits and other carbohydrate sources, which prevented some of the weight loss during the course, in particular loss of muscle proteins and glycogen reserves. From calliper measurements and needle biopsies the estimated fat loss is 3-4 kg during the course (Rognum et al 1982).

The cadets were normally not allowed any organized sleep during the course except for the cadets participating in a 7 day course who got 3-4 hours of sleep on day 5. From heart rate recordings, from wrist actigraphy (Vitalog) and from our own and officers' observation, the cadets' sleep during the course has been estimated to 1-3 hours totally.

The course takes place in the eastern part of Norway (60.8° North, 11.5° East) in a forest area at 500 m altitude. The courses organized in June (Papers 1, 7) normally had nice weather with temperatures between 20 and 30 °C during the day and between 5 and 15 °C by night. In September the nights were dark and with considerable variation in the weather and temperatures. The weather was nice during most courses, but we have also seen snowfall and 0 °C by night with cool and rainy days. In course I described in paper 5, the weather was warm and nice, whereas course II was cold with snowfall by night, and with cold and rainy days. Apparently this did not have any major influence on the steroid hormones investigated in spite of changes in the training program with lower physical activities by day, when the temperatures were high, and reduced activities by night in the course with snowfall and low temperatures. Most blood sampling and investigations were organized in the field or the cadets were taken to a military barrack (10-30 minutes drive) for medical and scientific investigations (bicycle experiment, GnRH-test). For more complicated investigation the cadets were brought to a near by hospital at Elverum (20-30 minutes drive) (Gastroscopy). For some investigations we had to drive for approximately 2 hours to Oslo to have the necessary specialist aid and laboratory facilities (Gastroscopy with laser dopler measurement of mucosal blood circulation).

2.2 Blood sampling

Blood samples were taken by venipuncture in the antecubital vein, whereas repeated sampling was performed trough an indwelling plastic canulla also in the antecubital vein. The blood was drawn into pre-chilled vacuum tubes containing the necessary anticoagulants, such as EDTA or heparin, or also substances necessary to conserve the hormones investigated, such as the peptidase inhibitor aprotinin. The blood for preparation of plasma was centrifuged immediately in a refrigerated centrifuge for approximately 20 minutes, and the plasma was frozen at -80 °C on dry ice. Blood for preparation of serum was allowed to clot at room temperature for 30 minutes before centrifugation. The samples

were kept at - 80 °C in an ultrafreezer until analyzed. If several hormones were to be analyzed in plasma from the same aliquot, the less stable hormone was analyzed first. All samples from both control and stress experiments were consistently treated in exactly the same way and stored at exactly the same temperature. All sample analyses from one person were run in the same assay with the same standards. If possible the whole experiment was analyzed in one assay and with the same standards. Most of the time the person performing the analyses did not know beforehand what was anticipated from the different experiments.

2.3 Biochemical analysis

Catecholamines in plasma were analyzed using a radioenzymatic method (s-adenosyl methionin as a tritiated methyl donor transferred to the amines by catechol-o-methyl transferase), which is a modification of the method of daPrada and Zürcher (1976) described in detail in Paper 2. Total concentration of catecholamines was analyzed with the same method after desulphatation of the sulphate group by a sulphatase (Sigma S-1629). The plasma concentration of the conjugated catecholamines was calculated by subtracting the concentration of the free amines from the total concentration of catecholamines in blood.

All the other hormone analyses were performed with radioimmunoassays with commercial kits, the methods and antibodies or radiolabeled tracers are described in the different papers.

Glucose was analyzed with three different methods, first the hexokinase method (Boehringer), then by an oxygen sensitive electrode (Beckmann Analyser 2), and lastly by the Kodak Ektakem DT2 system. Inter and intra assay coefficient of variations were normally calculated on the basis of the results presented.

2.4 Statistical analysis

Since our experiments often include serial samples from the same persons, such as every morning, or responses to different types of stimulation, we had to use an analysis of variance for repeated measures. The data were analyzed by means of commercial statistical programs such as SPSS (Manova) or BMDP (4V, 5V, 8V). If the requirements for normal distribution or equal variance were not satisfied, the results were transformed by logarithmic transformation or analyzed with non-parametric methods. However, since the non-parametric methods are rather few and without possibilities to treat many groups in a repeated analysis the parametric methods were preferred. Student's t-test has been used to identify significant differences.

2.5 Ethical considerations

The military ranger training courses started in Norway in 1967 with similar American military training courses as models. The military purpose for this training was:

- make the cadet fit as a leader in combat with high intensity for prolonged periods of time (6-7 days)
- by self-experience feel and see how their own and other cadets' performance is affected by factors relevant for combat situations
- be able to exert situational leadership also during combat operations
- learn coping strategies such as "overlearning" (drill) in order to sustain their performance
- train each of them to live and survive under extreme conditions in the field and to prevent long term health hazards.

However, after some years of such training at the Norwegian Military Academy, the physicians in the Norwegian Joint Medical Service, with their experience from World War II, found it necessary to give this training a close medical surveillance. They also took the initiative to start a research program to examine the soldiers' limits of tolerance and possible health consequences of such training, so that the courses could be run within safe limits. The purpose was also to train military physicians to be qualified to give medical support during training under extreme environmental conditions.

The mechanisms for mental and physical exhaustion were also important areas of research particularly their interaction with the recovery and survival of traumatized soldiers. The stress-factors responsible for decrement in mental and physical performance and countermeasures were investigated, such as need for sleep and food.

To avoid mixing the function as medically responsible and scientist, these two roles have been strictly separated. The person medically responsible will not participate in the research program or be author of papers from the research program. This is to secure that the medical responsible physician only bases his decisions on pure medical foundation.

The courses have been organized only for educational purposes. However, some standardization of the training conditions has been allowed, which also has protected the cadets against impulse alteration in the training program by newcoming officers. Alterations in the training program due to research purposes have been to give one group extra food, extra sleep etc. in order to compare performance between groups of cadets. In addition cadets have been taken out of the course for medical examinations or blood sampling, during which time the cadets got extra rest, sometimes to the embarrassment for

the officers' training program. The research program has been presented for and approved by the Norwegian Joint Medical Service.

All cadets participating in the research program gave their informed consent, and were free to withdraw from the program at any time during the course or in the control experiment without being subjected to penalties. The subjects of the investigations never withdrew from the program during the course. It has happened, however, a couple of times that cadets were prevented from participating in the control experiments, mainly because they had moved away from the Oslo area.

3 GENERAL DISCUSSION

3.1 The catecholamines and adrenergic receptors

Catecholamines are important regulators of homeostasis and are indispensable for adequate physiological responses to different environmental conditions and demands such as exercise, fasting, cold stress, surgery, and diseases (Vendsalu 1960, von Euler 1974, Innes and Nickerson 1975, Unger et al 1980, Åkerstedt and Gilberg 1983, Dunne et al 1984, Kuchel et al 1986, Pluto et al 1987, Del-Prato et al 1990, Fillenz 1990, Landsberg and Young 1992). The adrenal medulla is the only source for adrenaline, whereas noradrenaline has a dual origin. Two third of circulating noradrenaline derives from the sympathetic nervous system and the rest from the adrenals. In addition to a small adrenal secretion of dopamine, plasma dopamine derives mainly from the sympathetic ganglion interneurones where dopamine is assumed to be a transmitter (Yoneda et al 1985). Physical exercise stimulates the release of noradrenaline more than adrenaline, whereas the opposite is the case for mental stress and hypogycemia (von Euler 1974, Åkerstedt et al 1983).

The pressor effect of adrenal extracts was first shown by Oliver and Schäfer in 1895. The active principle was named adrenaline (epinephrine) by Abel in 1899 (for review see Hartung 1931, Hoffmann and Lefkowitz 1992a). Barger and Dale (1910) studied the pharmacological effects of a large series of synthetic amines with similar activities as adrenaline which were termed sympathomimetics. Some of these act directly on the sympathetic receptors and some also by stimulating the release of endogenous amines. The inactivation of catecholamines from the sympathetic nerve terminals is mainly presynaptic reuptake, only 10 % "leak" out into the general circulation and can thus be detected in plasma (Fillenz 1990, Keiser 1995). Plasma amines are mainly inactivated in the liver where they are methylated by catechol-o-methyl transferase (COMT) or/and by monoamine oxydase (MAO) to vanyl mandelic acid and to homovanyl mandelic acid (HVA) for dopamine. There was probably no alteration in the inactivation of plasma catecholamines during the course, since the postexercise (bicycle test) inactivation of the amines was unchanged (Papers I, II). This fits well with the fact that only minimal

alterations were found in the liver function tests such as the galactose test, and in liver enzymes (Øktedalen and Opstad unpublished, Opstad et al unpublished).

The role of the conjugated plasma catecholamines has been a matter of dispute. In humans the conjugated amines are mainly sulphated, and there are high concentrations of the phenol-sulpho-transferase in the liver, in the gastro-intestinal tract and in blood platelets, and the plasma levels of conjugated amines increase after ingestion of amine-rich food. Sulphatation might therefore be the body's protection against food amines which could, if they were absorbed unchanged, induce dramatic changes in blood pressure and pulse frequency and cause anxiety and catabolic stress (Crout and Sjoerdsma 1959, Davidsen et al 1981, Mielke and Strobel 1994). In Paper II it is shown that conjugation does not serve to inactivate catecholamines after short term exercise, since there is no increase in the conjugated amine levels in the recovery period after the bicycle exercise test. Since the cadets did not ingest any significant amounts of nutrients during the course, the only source for the increased plasma levels of conjugated amines during the course had to be sulphoconjugation of free circulating amines. Sulphoconjugation therefore serves to inactivate catecholamines during prolonged exercise. Conjugated amines do not serve as a source for the free catecholamines during exercise, since there was no decrease in the conjugated catecholamines simultaneously with the increase in the free amines. Large interindividual variations have been found for the plasma conjugated catecholamines, particularly for dopamine. During the ranger course this large interindividual variation disappeared, which indicates that the variations are due to the ingestion of food that contains different amounts of amines.

Ahlquist (1948) classified the adrenergic receptors as α and ß, based on the rank order of potency of the different catecholamines in the vascular beds. The receptors have further been classified into α_1, α_2, $ß_1$, $ß_2$, D_1 and D_2 adrenoceptors, based on ligand-binding studies and responses to synthetic agonists and antagonists (Lefkowitz 1979, Hoffman et al 1980, Hoffman and Lefkowitz 1980, 1992a, b, Motulsky and Insel 1982, Arner 1992). The α_1 receptor is postsynaptic and located on the effector tissues such as vascular smooth muscle. Stimulation of this receptor may cause vasoconstriction, increased peripheral vascular resistance and increased blood pressure, pupillary dilation and intestinal and urine bladder relaxation. The classic α_1-receptor agonist is phenylephrine, and its antagonist is prazosin. Its effects are mediated through the phosphoinositols and increase in cytosolic calcium. In contrast many α_2 receptors are located presynaptically and their stimulation inhibits the noradrenaline secretion. α_2-receptor stimulation decreases sympathetic nerve activity and causes aggregation of platelets (Keiser 1995). Metyldopa and clonidine are α_2 agonists and are used in the treatment of hypertension. The α_2 antagonist is yohimbine, and its effects are mediated through an inhibition of adenylate cyclase and activation of potassium channels (Fain and Garcia-Sainz 1980). $ß_1$-adrenoceptor stimulation causes positive inotropic and chronotropic effects in the heart, lipolysis and increased renin secretion by the kidney. Stimulation of the $ß_2$ receptor causes bronchodilation, vasodilatation particularly in the skeletal muscle, glycogenolysis, smooth muscle relaxation

and increased release of noradrenaline from sympathetic nerves. Adrenaline is a much more powerful stimulant of the β_2 receptor than noradrenaline, whereas they have approximately equal potency at the β_1 and α_2 receptor. For the α_1 receptor, noradrenaline is the most powerful stimulator. Dopamine is a weak stimulator of the α and β receptors, and in addition it has its own receptor, the D_1 receptor, which induces vasodilatation in the coronary, renal, mesenteric and cerebral vascular beds. Stimulation of the D_1 receptor in the kidney causes natriuresis and diuresis. This may be a mechanism for the natriuresis and diuresis during the first days of starvation. D_2-receptors are located presynaptically in the sympathetic nerve endings, and their stimulation inhibits release of noradrenaline and sympathetic ganglion transmission. Stimulation of the dopamine receptors in the brain causes emesis and inhibition of prolactin release. The decreased prolactin levels during the course from rather high precourse levels may be due to dopamine inhibition of prolactin secretion. Both dopamine receptors mediate their effects through the adenylate cyclase. With high catecholamine levels for prolonged periods of time, such as in patients with pheochromocytoma, a desensitization may occur by several mechanisms; internalization of receptors, decreased binding affinity of the receptor on the cell surface, uncoupling of the receptor and decreased sensitivity of the adenylate cyclase activity (Fraser et al 1981, Snavely et al 1982, Keiser 1995). The present investigation demonstrates an adrenergic desensitization during prolonged stress in that both pulse rate and blood pressure and their responses to short term physical exercise are almost unchanged in spite of considerably increased plasma catecholamine responses (Opstad et al 1980, Papers I and II). This is well in accordance with the decrease in the leukocyte adrenergic receptors during the course (Paper III). The high correlation between increased plasma catecholamines and reduced number of adrenergic receptors indicates a homologous down-regulation during the course. In contrast, the increased number of adrenergic receptors at the end of the course might be explained by a heterologous upregulation, because corticosteroids, which are increased during the course, are known to stimulate the synthesis of adrenergic receptors. Thyroid hormones are also known to stimulate the synthesis of adrenergic receptors (Williams et al 1977, 1979, Davies and Lefkowitz 1980, 1981, Ginsberg et al 1981) which should have given the opposite results, since all thyroid hormones decrease during the course (Opstad et al 1984). Another mechanism which may explain the increased cateholamine levels, is decreased reuptake into sympathetic nerve terminals, which is the main mechanism for noradrenaline inactivation. Decreased reuptake in sympathetic nerve terminals is probably the reason for increased noradrenaline responses to stress in aged people (Esler et al 1995).

Paper III shows that the adrenergic desensitization during the course is not only due to reduced number of adrenergic receptors, but is also due to reduced adenylate cyclase activity, in that the cAMP response to adrenaline stimulation was reduced during the course, both in its sensitivity and its maximal response. The adrenergic desensitization seems to be due to the prolonged physical exercise, since there was minimal effects of sleep or food deprivation on the catecholamine responses to bicycle exercise during the course. One explanation for this is that the effect of exercise dominated over a small effect

of food or sleep deficiency. But this also shows that there can not be any potentiation between these effects. However, there was a surprisingly small effect of glucose infusion on the plasma catecholamines during a bicycle exercise test (Paper III), since it is known that particularly adrenaline is very sensitive to decreased plasma glucose levels (von Euler 1974). High stress levels and associated hormones have traditionally been associated with unfavourable survival rate. More recently it has been shown in a population study that low resting plasma adrenaline levels were associated with an unfavourable survival rate (Christensen and Jensen 1994).

The increased catecholamine responses to bicycle exercise shows that there was no sign of exhaustion in the sympathoadrenal nervous system during the course, and that the mechanism for decreased performance is to find in a desensitization of the peripheral tissue to nervous and hormone stimulation. During prolonged stress with high turnover in the sympathoadrenal system it has been proposed to give supplements of tyrosine, the precursor in the synthesis of catecholamines, to prevent exhaustion. There was no indication of such a need during the ranger course of 5-7 days since there was no deficiency in the cadets' catecholamine response to exercise (Ahlers et al 1994, Liebermann 1994).

Several investigations have shown that plasma levels of catecholamines show a circadian rhythm with the highest levels during daytime and the lowest levels at night (Åkerstedt and Gillberg 1983, Smolensky and D'Alonzo 1992, Touitou and Haus 1992) . Most of these investigations were based on urine measurements with the subjects recumbent. In order to have similar experimental conditions at day and at night in the control and recovery experiments, the subjects had to rise from their beds and walk for approximately 5-10 minutes to a classroom and rest for 10-15 minutes before blood sampling in the sitting position. With these experimental conditions, with a standardized level of activation of the adrenergic system, a small circadian rhythm might have been hidden. However, it might also be concluded that this possible circadian rhythm must be very small and may be explained by different level of sympathetic activation, independent of a proper endogenous circadian rhythm (Paper VII). In addition the increased free urine catecholamines may more reflect increased renal sympathetic activity than plasma catecholamine levels. The fact that some investigators has found a more pronounced circadian rhythm for adrenaline then for noradrenaline may be due to a diffusion of cortisol from the adrenal cortex to the adernal medulla and the stimulation of N-methyl transferase by cortisol (Holmboe et al 1975, Åkerstedt and Frøberg 1978, 1979, Åkerstedt et al 1983).

Catecholamines normally do not pass the blood-brain barrier since they are rather polar substances; noradrenaline is the most polar, closely followed by adrenaline, whereas dopamine is the less polar. The possible action of plasma catecholamines in the CNS therefore has to be through locations where the blood brain barrier is fenestrated. Most often this is in the basic parts of the brain, particularly in the hypothalamus. However, there is no information on possible effects of circulating catecholamines on the CNS. During an

adrenaline or noradrenaline infusion test lasting for 20 minutes during the ranger training course a striking clinical difference was observed between the two hormones, in that the subjects given adrenaline became very alert, and stayed awake in spite of prolonged sleep deprivation. This was not the case for the subjects given noradrenaline who got drowsy and tended to fall asleep all the time. The difference was less clear during the control experiment. This difference might have several explanations. There might be a direct effect of adrenaline and not of noradrenaline in the CNS. Another explanation could be that the difference is due to peripheral factors such as the different effect of adrenaline and noradrenaline on glucose mobilization. However, if this effect was mediated via increased plasma glucose levels, the same effects should have been observed in subjects given glucose intravenously. Since adrenaline does not pass the blood-brain barrier, it is tempting to suggest that this effect is due to secondary mechanisms. In the brain, noradrenaline serves as neurotransmitter for the neurones in the locus coeruleus, which have a widespread distribution of their fibres through most of the brain. In contrast, dopamine has a more limited distribution mainly in the nigro-striatal pathway and some interneurones in the hypothalamus. Adrenaline is not thought to have any significant role in the CNS, but may play a role as transmitter in the spinal cord or in sympathetic interneurones (Frankenhaeuser 1971, Joëls and De Kloet 1989, Landsberg and Young 1992).

3.2 Adrenal steroids

Thomas Addison discovered in the mid-1800's that the adrenal cortex is absolutely vital for survival. A century later it was discovered that the adrenals had two hormones that were necessary for survival, a glucocorticoid and a mineralocorticoid.

The hypothalamo-pituitary-adrenocortical activation was considered by Selye as a main physiological reaction to stress with the following shrinkage of the thymus, spleen, lymphatic structure and deep bleeding ulcers in the stomach and upper gut. The anti-inflammatory effects of the glucocorticoids were in opposition to the disease of adaptation of Selye and were therefore not recognized for decades (Selye 1946, 1950, Ritchie and Nemeroff 1991, Munck and Náray-Fejes-Tóth 1995).

The adrenal cortex can be divided in 3 separate zones, the zona glomerulosa which produces the mineralocorticoids, zona reticulosa which produces the glucocorticosteroids and zona fasciculata which produces the adrenal androgens (Parker et al 1987, Parker 1989, 1995). There are no sharp distinctions between the different zones, and in addition there is considerable crossreactivity in their physiological effects, and there is to some extent an interconversion of the different steroids in the peripheral tissues, particularly in the liver and in fat tissue but lately also demonstrated for muscle tissue. The mineralocorticoids are mainly regulated by the renin-angiotensin axis, but are also stimulated by ACTH. Glucocorticoids are almost exclusively regulated by ACTH, whereas adrenal androgens beside ACTH may also stimulated by a polypeptide isolated from the pituitary that is

different from ACTH, however, this is still under debate (Odel and Parker 1980, Vermeulen 1983, Parker et al 1987, Parker 1995).

The 4-5 fold increase in the plasma levels of aldosterone during the course is mainly due to reduced intake of food and by that the intake of NaCl. However, NaCl/K excretion is strongly regulated, and by a 90 % decrease in the urine excretion of NaCl, plasma levels of NaCl were maintained constant. When extra food was given containing approximately 20 g/24h of NaCl for each cadet, both plasma renin activity and aldosterone levels were reduced by more than 50 %, but still the salt excretion from the kidneys was reduced by 50 % compared to normal (Opstad et al 1985b, Opstad et al 1994). Clinically only a few cadets showed symptoms of salt deficiency, and only in connection with exercise and high environmental temperature. Therefore, in spite of the very strong regulation of salt balance by the kidney, occasionally small extra challenge was sufficient to cause clinical symptoms of hyponatremia.

Glucocorticoids are secreted from the adrenal gland into the blood stream and have profound effects on almost all physiological functions. The glucocorticoids pass the cell membrane, bind to and activate the glucocorticoid receptor proteins which are localized intracellularly in the cytoplasm and further activate the nucleic acids in the nucleus of the cells.

The increase in glucocorticoids during the course is due to a combination of physical exercise and energy deficiency. During the bicycle exercise test the plasma cortisol levels were lower both during the exercise test and during recovery in the well fed subjects. To rise the plasma levels of cortisol in rested subjects the exercise has to last for at least 60 minutes and at more than 60 % of maximal oxygen uptake (Sundsfjord et al 1975). However, during the course this rise starts earlier and at lower exercise intensity (Opstad et al 1980, Paper I). It is well established that cortisol stimulates energy mobilization at many levels. First of all cortisol stimulates gluconeogenesis through the stimulation of relevant hepatic enzyme systems. In addition cortisol mediates its action through the stimulation of the synthesis of adrenergic β-receptors, making adrenaline more efficient. Thereby cortisol counterbalances the stress-induced homologous downregulation of the β-receptors and is an important mechanism for preventing adrenaline from loosing its efficiency during prolonged exhausting stress. This will contribute to maintain physical as well as mental performance capacity. Cortisol may also contribute during prolonged physical strain to minimize all inflammatory processes which might be painful and which might prevent soldiers from performing their tasks. Cortisol passes the blood brain barrier and influences a number of brain functions such as mental performance and memorization (Funder 1991). The antiinflammatory response may sometimes even be lifesaving, since it prevents the inflammatory process from being harmful for the body.

In contrast to glucocorticoids and mineralocorticoids, which increase during the training courses, there is a decrease in the adrenal androgens such as dihydroepiandrosterone,

androstendione and 17α-OH progesterone. ACTH is known to stimulate the adrenal secretion of all steroids. The decrease found for ACTH levels could then well explain the decreased secretion of andrenal androgens. However, ACTH levels measured do not necessarily reflect the mean level of ACTH stimulation of the adrenals. To avoid testing the acute effects of exercise, but rather a steady physiological state, the cadets were not allowed any significant exercise just before testing or blood sampling. The problem in this context is that the subjects during the course are rarely in a steady state situation but are rather in a state of activation or in a state of recovery. In the present case ACTH, which has a short half life of only some minutes, therefore recovers faster then cortisol, which has a half life of approximately 90 minutes. During this period cortisol will, in addition, exercise a negative feedback on ACTH production which is stronger than normal and by consequence lead to lower plasma ACTH levels than normal. So in spite of the measured ACTH levels, ACTH may well be responsible for the increased cortisol levels. ACTH will also contribute to the increased aldosterone levels but in combination with an even stronger and more important regulator, the renin-angiotensin system (Parker et al 1987, Parker 1995). In contrast to the free adrenal androgens, the sulphated form, dihydroepiandrosterone-sulphate (DHEA-S) which circulates in the plasma in micromolar concentration and with a half life of several days, increases during the course. This is probably due to increased secretion from the adrenals and therefore shows that the adrenal gland may differentiate its secretion of the different androgens or steroids. The increased levels of DHEA-S might also originate from peripheral sulphatation of the free androgens, or there may even be a combination of the increased secretion and peripheral sulphatation of free androgens. Our original hypothesis that increased adrenal androgens could compensate for effects of decreased testicular androgens is not verified. This is also supported by the hypogonadic clinical symptoms during the course such as almost no beard growth, reduced muscle strength and less aggressive behaviour. It is presumed that a main reason for the decreased adrenal androgens during the course is the physical strain particularly during night time. However, the decrease of unconjugated adrenal androgens found during sleep deprivation by Åkerstedt et al (1980) may indicate that also sleep is important to preserve the increase in adrenal androgens during night time.

Like all other free steroids, the glucocorticoids pass the blood-brain barrier and are known to influence behaviour, mood, neuronal excitability and electrical activity. Behavioural changes are observed both in excess states such as Cushing's disease and in deficient states such as Addison's disease. Sleep disorders are often associated with glucocorticoid therapy (McEwen 1979). Adrenalectomy leads to the loss of neurones in the hippocampal formation, particularly in the dentate gyrus (Sapolsky et al 1991), whereas very high levels of glucocorticoids have been shown to cause the death of hippocampal CA3 pyramidal cells and to potentiate neuronal death evoked by toxic substances (Packan and Sapolsky 1990, Stein-Behrens et al 1992, Munch and Náray-Fejes-Tóth 1995).

Glucocorticoid receptors are widely distributed in neurones and glial cells throughout the brain (Funder 1991, Power et al 1991, Laudet et al 1992), whereas the mineralocorticoid

receptor is mainly localized in the hippocampus and septum. In spite of the fact that mineralocorticoid receptors have a lower affinity for the glucocorticoids than for aldosterone, this is compensated by far higher concentrations of glucocorticoids. There are small areas where the mineralocorticoid receptor is protected against glucocorticoid effects by 11β-hydroxysteroid dehydrogenase and is by that aldosterone selective. In the limbic structure mineralocorticoid receptors mediate glucocorticoid effects. Studies in hippocampal slices have shown that low concentrations of glucocorticoids, when only the mineralocorticoid receptors are activated, give enhanced neuronal excitability. In contrast, high concentrations which activate the glucocorticoid receptors, suppress hippocampal excitability (Jöels and De Kloet 1989, Kerr et al 1989, Trapp et al 1994). In addition to the electrophysiological effects, glucocorticoids inhibit glucose transport in hippocampal neurones and glial cells, they affect glycerol-phosphate dehydrogenase (McCarthy and deVillis 1980) and glutamine synthetase in astrocytes (Hellermayer et al 1981) and induction of K^+ channel mRNA synthesis and channel expression in pituitary cells (Levitan et al 1991).

Also the adrenal androgens pass the blood brain barrier, but in contrast to the glucocorticoids, mineralocorticoids and testosterone, the cerebral concentration of pregnenolone, DHEA and their sulphate and fatty esters is considerably higher than in plasma. In addition the sulphate and fatty acid esters do not cross the blood brain barrier, and it has been shown that their variations are independent of the plasma variations. It is also shown that the oligodendrocytes have the enzymes (Cytochrom P-450) necessary to convert cholesterol to Δ5-3β-OH androgens and their conjugated and lipoid derivatives. Moreover, DHEA and its sulphate persisted for several weeks after pharmacological or surgical glandular suppression. This contrasts with testosterone, glucocorticoids and mineralocorticoids which disappear in the brain after the removal of their respective glands, and which normally have lower concentrations in the brain than in plasma (Corpéchot et al 1983, Denner et al 1990, Akwa et al 1991, 1992, Robel et al 1991, Vourch et al 1992). As for plasma steroids, brain steroids show a rather strong circadian rhythm with the highest levels during the dark period. The acrophase of corticosterone in plasma preceded the acrophase of brain DHEA and pregnenolone, indicating an independence between plasma and brain steroids. DHEAS has been shown to interact with rat forebrain membrane γ-amino butyric acid (GABA) receptor complex as a non-competitive negative neuromodulator. The GABA receptor is an oligomeric protein complex that, when activated by an agonist, produces an increase in neuronal membrane conductance to Cl^- ions, resulting in membrane hyperpolarization and reduced neuronal excitability (Chavatal and Kettenmann 1991, Demirgoron et al 1991, Robel et al 1991). Thus, adrenal androgens cause neuronal excitation and regulate neuronal and glial growth *in vitro* (Carette and Poulain 1984, Bologna et al 1987, Muntwyler and Bologna 1989), and also affect memory and aggressive behaviour in mice (Flood and Roberts 1988, Young et al 1991, Flood et al 1992). Pregnenolone, DHEA and DHEAS have also been found in the peripheral nerve tissue and might be trophic factors for these nerves (Akwa et al 1991, Chvatal and

Kettenmann 1991, Demirgoron et al 1991, Morfin et al 1992). Nasman et al (1991) have shown that plasma DHEAS was decreased in patients with Alzheimer's disease. Morris et al (1987) have shown that concentration of gonadal and adrenal androgens is related to female libido. Plasma adrenal androgens show a peak concentration in the third decade of life, and then decrease gradually to very low levels in senescence. A decrease is also found for gonadal androgens with ageing with considerable interindividual alterations, however, the decrease is far less pronounced than for the adrenal androgens (Zumoff et al 1982, Davidson et al 1983, Tenover et al 1987a, b, 1988, Swerdloff and Wang 1993a, b, Winters 1995). In contrast to the androgens, the classical stress hormones, the glucocorticoids, the catecholamines, and the other counterregulatory hormones increase with age (Landsberg and Young 1992, Munck and Náray-Fejes-Tóth 1995). It has been speculated wheather the hormonal alterations may be one of the mechanisms behind the process of ageing. In this case the demonstrated alterations found during stress will promote the process of ageing. However, since "adrenal androgens" probably do not have the same source in brain and plasma, and since we and others have not investigated the effect of stress on brain androgens, we do not know exactly the possible consequences for the central nervous system of alterations in these hormones (Meites 1991).

3.3 Testicular androgens

The testicular androgens are steroids that are responsible for the development of the male phenotype. They have three main effects: stimulation of masculine sexual characteristics, anabolic function by stimulating the increase of muscle mass, and influence on behaviour, particularly by stimulating initiative and aggressiveness. Plasma testosterone derives for 95 % from the testis, and is its most important and potent androgenic hormone. The rest (5%) derives from conversion of androgen precursors to testosterone in peripheral tissue and also for a very small part from direct adrenal secretion (Catlin 1995, Handelsman 1995, Hiipakka and Liao 1995, Kretser et al 1995).

Androstenedione and dihydroepiandrosterone are also secreted from the testis but at rather low rate. Their biological effects are small, but they may serve as precursors for the peripheral synthesis of testosterone or oestrogens. The androgens affect the development, growth and function of a wide variety of tissues and cell types by their interaction with the intracellular androgen receptor. Androgen-receptor complexes bind to specific sequences of DNA and modulate the rate of specific gene transcription (Kretser et al 1995). The biological effects of androgens in different tissues are determined by the tissue concentration of androgen receptors and also by the tissue concentration of the enzyme 5α-reductase which converts testosterone to dihydrotestosterone which has a 5 times higher affinity for the androgen receptor than testosterone. Tissues containing androgen receptors include the reproductive organs, brain, kidney, liver, skin, skeletal muscle, cardiac muscle, bone, larynx, thymus, hematopoietic and lipid tissue. Although a small portion of 5α-dihydrotestosterone (DHT) is secreted from the testis, most of the circulating DHT derives

from peripheral metabolism of testosterone in various tissues. The tissue sensitivity to androgenic hormones is also dependent on the tissue content of 5 α-reductase, which is necessary for the conversion of testosterone to dihydrotestosterone, since this enzyme may convert androgens to the most potent androgen DHT (Hiipakka and Liao 1995).

During the ranger training course there is a 90 % decrease in the plasma levels of both free and total testosterone and dihydrotestosterone. The nocturnal increase in the plasma levels of testosterone was completely abolished during the course, showing that night activity is even more deleterious for anabolism and recovery than day activity (Aakvaag et al 1978 a,b,c, Opstad and Aakvaag 1982, 1983, 1985, Papers V, VI, VII).

The decrease in dihydrotestosterone probably reflects the decrease in androgen precursors since the percent decrease in testosterone and dihydrotestosterone are similar. From the present results we do not have any indication of alterations in the 5α-reductase activity in androgen target tissues during the course.

The decrease in testicular androgens was mainly due to the physical strain since no significant effect was found when the cadets were given extra food. A slower decrease was seen in subjects given 3 hours of extra sleep each night, however, all cadets reached the same level on the last day of the course (Opstad and Aakvaag 1982, 1983, Elias and Wilson 1993). Others have shown that extra food might reduce the decrement shown to take place for testosterone during a military training course (Guezennec et al 1994). Similarly two British explorers to the South Pole who had a strenuous travel on ski, who pulled their sleigh weighing approximately 200 kg for 12 hours each day with an energy deficiency leading to a weight loss of 16 kg body weight, also showed a dramatic decrease in testosterone levels (Mike Stroud, personal communication). It is also shown in rats that fasting leads to decreased secretion of LH and testosterone. Hypothalamic LHRH, pituitary LH and FSH were unaffected, indicating an inhibition of LHRH during the fasting (Badger et al 1985). A possible explanation for the difference in effect of food in our course compared to the study by Guezennec et al (1994), is that our course probably is harder and that the exercise induced decrease in testosterone was maximal independent of the other stressfactors. The present papers also show that the decrease in testosterone is due to reduced secretion of LH/FSH. Further the increased LH/FSH response to GnRH stimulation indicates that there is a reduced hypothalamic GnRH secretion during the course leading to an increased sensitivity of the LH/FSH producing cells to GnRH stimulation. This shows that androgen secretion during the course is regulated from the hypothalamus and its inputs from other brain areas.

The clinical signs of reduced androgen activity are present since the beard growth during the whole course corresponds to a normal growth of one day, and this beard growth takes mainly place during the first day of the course. This corresponds well to the alterations in the androgen hormones. During the course the cadets become less aggressive, show less

initiative, become more defensive and depressive, which is also in accordance with the alterations in the plasma levels of androgens (Opstad et al 1978, Myhrer 1987).

Like other steroids, testosterone and dihydrotestosterone may cross the blood brain barrier and bind to cerebral androgen receptors. Androgen receptors are mainly localized in the medial preoptic area, bed nucleus of the stria terminalis, amygdala, hippocampus, thalamus and several hypothalamic nuclei including the periventricular nuclei, supraoptic, and ventromedial nuclei and median eminence. In addition there are androgen receptors in other areas of the brain such as the frontal cortex etc., but these areas have lower receptor densities than the classical sites (Wortsman et al 1987, Sar et al 1990, Jones and Pfaff 1991, Takeda et al 1991, Genazzani et al 1992, Burgess and Handa 1993, Menard and Harlan 1993, Clancy et al 1994). In addition androgens may act through the oestrogen receptor since aromatase irreversibly transforms testosterone to oestradiol and androstendione to oestrone. This enzyme was originally found in ovary and placenta, but has also been localized to the mammalian brain particularly in the medial preoptic area, septal region, the bed nucleus of the stria terminalis and the tuberal hypothalamus (Balthazart and Foidart 1993, Hutchison 1993, Roselli and Resko 1993). The "limbic-telencephalic" aromatase-immunoreactivity is shown to be independent of gonadectomy, whereas the hypothalamic aromatase-immunoreactivity disappears after gonadectomy (Jakab et al 1993). Occupation of oestrogen receptors in the male brain is dependent on brain aromatase activity, whereas the occupation of oestrogen receptors in the female brain is more dependent on circulating oestrogen particularly during the preovulatory oestrogen surge. In addition both oestrogen and androgen receptor concentrations decrease after gonadectomy and reappear after substitution and are further increased by anabolic steroid abuse (Sar et al 1990, Takeda et al 1991, Menard and Harlan 1993, Catlin 1995). Dihydrotestosterone has a 4-5 times higher affinity for the androgen receptor than testosterone, and tissue sensitivity for testosterone may therefore be quite dependent on the tissue concentration of 5 α-reductase which converts testosterone to dihydrostosterone. Tissues such as the external genitalia and accessory sex glands have high concentrations of 5 α-reductase, and congenital deficiency in this enzyme causes female external genitalia. However, the significance of this enzyme in the brain sensitivity for androgens is less investigated. In contrast, aromatase has been shown to be necessary for the sexual differentiation of the fetal brain in rats and also for the adult (Kalra and Kalra 1991, Swerdloff et al 1992, Hutchison 1993, Jacab et al 1993). Abuse of anabolic steroids promote aggressiveness and motivation which might be an important contribution to increased performance (Moritani and DeVries 1979, Catlin 1995). A positive correlation has also been found between aggressiveness and blood testosterone levels during puberty, and adulthood in prison population, adolescent boys and military veterans (Hines and Green 1991). Testosterone is also shown to decrease with ageing, and some look upon aged men as androgen deficient and believe that reduced testosterone levels are responsible for asthenia, decreasing muscle mass, osteoporosis, and decreased sexual activity (Swerdloff and Wang 1993a, b, Winters 1995).

The protein wasting during the course is probably also enhanced by the decrease in the plasma androgens. However, in contrast to the decreased beard growth, which is a rather specific androgenic effect, the protein wasting and alteration in behaviour are not specific and therefore have additional explanations. Although sleep deprivation affects testosterone secretion, there are a multitude of other mechanisms that are responsible for the behavioural consequences of sleep deprivation, and the decrease in testosterone is only one of them. The most important reason for protein wasting is probably the state of fasting during the course with lack of carbohydrates and proteins and an extremely high and continuous need for energy. Although there was no significant effect of fasting on plasma androgens during the course, it has been shown by others that fasting may affect plasma androgen levels. It has been speculated whether the cadets' combat performance, such as aggressiveness, initiative and muscle strength could be improved by giving the cadets androgens. However, the "wisdom of the body" might indicate that high androgen activity is incompatible with a high energy production. Adrogens in such extreme conditions could disturb this mechanism and force the body to take the energy from other more critical sources for survival than the tissues containing androgen receptors. This might be a very hazardous experiment. In contrast, androgens could probably ameliorate or shorten the cadets recovery period if the androgen substitution is combined with an adequate diet.

3.4 Thyroid hormones

Thyroid hormones have a myriad of physiological functions and induce alterations in almost all metabolic pathways and organs (Dumont and Vassart 1995, Jameson and deGroot 1995, Nicoloff and LoPresti 1995, Refetoff and Nicoloff 1995, Sarne and Refoteff 1995). Thyroid secretion is mainly regulated by thyroid stimulating hormone (TSH) through the hypothalamo-pituitary axis (Wilber 1995). Thyroid hormones increase oxygen consumption, affect protein, carbohydrate, lipid and vitamin metabolism. These hormones also interact with a number of other hormones, peptides and growth factors so that many of their effects occur trough interaction with other endocrine systems. The main effects of thyroid hormones on metabolism and cellular differentiation, development and growth are closely interrelated and represent a complex integration of pathways both at the cellular level and in terms of whole body physiology. Many of the developmental effects are not reversed by later treatment with hormones, suggesting that thyroid hormones act in combination with other differentiation factors that may not be available later in life. Clinically alterations in thyroid hormones were long the basis for the measurement of basal metabolic rate or oxygen consumption which are increased in hyperthyroidism and reduced in hypothyroidism. Measurement of oxygen consumption in individual tissues has shown that the metabolic effects of thyroid hormones on oxygen consumption are highly variable in different organs and tissues with marked effects in the heart, skeletal muscle, liver, kidney and gastrointestinal organs, whereas the brain, spleen and gonad tissues are metabolically less responsive. The pituitary gland shows paradoxical response since there is increased metabolic activity in hypothyroidism and reduced activity in hyperthyroidism.

These variable tissue responses are partly due to tissue presence of receptors for thyroid hormones. Oxygen consumption is, however, not a marker of thyroid hormone effects in all tissues. This is for instance the case for thyroid hormone effects in the brain which shows one of the most pronounced clinical effects of hypo- and hyper-thyroidism. Most of the effects of thyroid hormones are now considered to occur through the actions of nuclear receptors that cause alterations in gene expression. The thyroid stimulation of energy production also leads to increased heat production which will ameliorate the cadets' cold tolerance (Jameson and deGroot 1995). During the ranger course there is an increase in oxygen consumption both at rest and during work in spite of the decreased levels of thyroid hormones (Bahr et al 1991).

All plasma thyroxin (T4) derives from thyroid secretion, whereas only 5-10 % of T3 and 1-3 % of rT3 derive from thyroid secretion. The rest originates from peripheral conversion of T4, mainly in the liver. Only 0.02 % of T4 and 0.3 % of T3 circulate in the free form, the rest is bound to plasma proteins such as thyroxin binding globulin (TBG), thyroxin binding prealbumin (TBPA) and albumin. Nuclear receptor saturation of 75 % in the brain and pituitary and 50 % in liver and kidney in spite of a plasma concentration of $2x10^{-11}$ M for T4 and $6x10^{-12}$ M for T3 versus a dissociation constant for the thyroid receptor of $2x10^{-9}$ M for T4 and $2x10^{-10}$ M for T3 indicates active transport mechanisms across plasma membranes. This is supported by the fact that for instance the concentration of T3 is 50 times greater in the erythrocytes than in plasma (Osty et al 1990, Nagashima et al 1993, Jameson and deGroot 1995).

Clinical observation of the cadets during the ranger training course showed that all had symptoms of hypothyroidism, since they shivered, were easily freezing, had slow motions and were also mentally slower than normal. The thyroid studies performed showed a decrease in thyroid hormones corresponding to the half life of T4 (Aakvaag et al 1978a, b, Opstad and Aakvaag 1981, 1983). An initial increase during the first day (12 hours) of activities was due to exercise, whereas the following decrease corresponding to the half-life of T4 was due to energy deficiency. The plasma concentration of rT3 also increased during the first day of activities due to exercise, but continued to increase during the course due to energy deficiency. This finding is well in accordance with the decreased plasma levels of thyroid hormones during fasting or starvation (Carlson et al 1977, Palmblad et al 1977, Jung et al 1980). There were no corresponding alterations in the plasma levels of thyroid stimulating hormone (TSH) during the course. Surprisingly the cadets that were allowed 3 hours of sleep each night showed the strongest decrease in TSH, whereas energy deficiency, which caused the difference in thyroid hormones, caused only moderately higher TSH levels. This is well in accordance with later published data on the inhibitory effect of sleep on TSH (Opstad et al 1984, Parker et al 1987).

Most hormones show circadian rhythm. The long half-life of thyroid hormones will mask a possible circadian rhythm. In the present work no significant circadian rhythm was demonstrated for thyroid hormones in spite of the presence of a circadian rhythm for TSH.

The circadian rhythm of TSH showed a maximum level at midnight before the other hormones and the lowest level in the afternoon. In addition to decreased TSH levels in plasma, prolonged continuous stress also gave an extinguished circadian rhythm, which was re-established after 4-5 days of recovery. In light of the present results one might believe that some deterioration of mental and physical performance might be due to alterations in thyroid hormones and that these alterations might be reversed by adequate food supply during the course (Pasquini and Adamo 1994). There are indications that the conversion of T4 to T3 in the liver is dependent on carbohydrate metabolism, and that optimal nutrition during prolonged physical strain must contain a certain critical amount of carbohydrates to maintain thyroid hormones at a sufficient level in order to preserve mental and physical performance, and in our climate preserve the soldiers' cold tolerance.

The cadets' hypothyroidism may contribute to the explanation of many of the alterations in both mental and physical function during the course, since hypothyroidism may lead to slowing of all movements and mental function, decreased alertness and vigilance, loss of ambitions and impaired memory. There may be cognitive impairment which may reach dementia. Hypothyroid patients often sleep longer than normal, may become anxious and depressed (myxedema madness) (Swanson et al 1981). Speech is slow, hesitant and hoarse, and physical movements are clumsy. Contraction and relaxation phases of reflexes are prolonged. Paresthesia, sensorimotor neuropathies, cerebellar dysfunction, ataxia, intention tremor and nystagmus may also appear but are reversible when thyroid hormone levels are normalized (Swanson et al 1981, Beghi et al 1989, Osterweil et al 1992, Utiger 1995). Myalgia, muscle cramps, muscle stiffness, weakness and increased fatigability are common (Kahleeli et al 1983), and pseudohypertrophy and pseudomyotonia of the muscles may develop with increased plasma levels of serum creatin kinase, lactate dehydrogenase and aminotransferase. Muscle fiber enlargements with oedema, loss of striation and sarcoplasmic degeneration, arthralgia, and joint stiffness due to synovial thickening are also described (Khaleeli et al 1983, Utiger 1995). The decreased thyroid function during the course may also contribute to impaired heart and lung function and to the gastrointestinal symptoms in the form of nausea, vomiting, decreased intestinal motility with constipation and abdominal distension (Ladenson et al 1992, Utiger 1995). The hypothyroidism may also contribute to the decreased haemoglobin levels during the course (Lindemann et al 1978, Tachman and Guthrie 1984) and may cause increased bleeding time, decrease in clotting factors and abnormal platelet function (Rogers et al 1982). The overall morbidity or mortality is not increased in hypothyroid patients, although some postoperative complications are more frequent, such as hypotension, cardiac failure, gastrointestinal dysfunction, and drug clearance is prolonged (Weinberg et al 1983, Ladenson et al 1984, Drucker and Burrow 1985).

3.5 Insulin and glucose metabolism

Glucose homeostasis is important for both human mental and physical performance. Plasma glucose is therefore strongly regulated by a variety of hormones. Insulin is, however, the only hormone able to reduce plasma glucose concentration, whereas a multitude of hormones may increase plasma glucose levels. These hormones are called the counterregulatory hormones and are the catecholamines, glucagon, human growth hormone, glucocorticoids and peptides such as VIP (for review see Vranic et al 1984, Cryer 1992, Morley et al 1992, Kahn and White1995, Polonsky and O'Meara 1995). Plasma glucose is regulated both by a direct effect of glucose and its metabolites in the β-cells of the pancreas and via its influence on the hypothalamic structure via the autonomic nervous system. The neurotransmitters influencing the β-cells of the pancreas are acetylcholine, noradrenaline, GABA, and different peptides such as somatostatin, VIP, etc. Also circulatory catecholamines may influence insulin secretion from the pancreas since the β-receptors stimulate and α-receptors inhibit insulin secretion from the β-cells (Keiser 1995). At high catecholamine concentrations the α-receptor dominates over the β-receptor and this might be one of the factors explaining the decrease of plasma insulin during exercise. During a bicycle exercise test, plasma glucose was shown to increase, whereas a decrease was seen during the same exercise test during the ranger course (Opstad et al 1980, Rognum et al 1981, Opstad 1987b). This is probably explained by the depleted glycogen depots and that the glyconeogenesis is too slow to compensate for the absence of muscle and liver glycogen during the course.

An impaired glucose tolerance was also observed during the course mainly due to the physical strain, whereas extra sleep or extra food did not reverse the impaired glucose tolerance (Fonnum and Opstad 1983, Opstad unpublished). The mechanism for this decreased glucose tolerance is a combination of lower insulin responses in combination with peripheral insulin resistance. The insulin response to glucose is normalized within 3-5 hours, whereas the peripheral insulin resistance subsides well beyond this time. The decreased insulin response to glucose is not due to adrenergic inhibition of the insulin secretion since α-blockers did not reverse the decrease in insulin secretion (Opstad unpublished). The practical consequences of these findings is that the cadets during or after the course are advised to eat small meals particularly as carbohydrates are concerned.

3.6 Pituitary hormones

Human Growth Hormone (hGH) stimulates protein synthesis as well as energy mobilisation. Energy mobilisation favours lipolysis, whereas the uptake of glucose in working muscles is inhibited by hGH, probably by decresing the insulin sensitivity. This effect of hGH is increased during starvation (Møller et al 1993). This also leads to an increased insulin response to glucose ingestion and impaired glucose tolerance. These changes may, however, also be mediated by the increased levels of plasma free fatty acids

stimulated by hGH. Increased hGH levels may therefore contribute to the glucose intolerance observed during the course. The rapid metabolic actions of hGH, such as lipolysis, promotion of glucose and amino acid transport across membranes, are probably mediated trough the hGH receptor directly. In contrast, many of the growth promoting actions of hGH are mediated through the intermediate action of insulin growth factor 1 which is mainly synthesised in the liver (Thorner et al 1992, Yarasheski et al 1992, Zenobi et al 1992, Clemmons 1993, Eriksen et al 1993, Gertner 1993, Haymond et al 1993, Vanderschueren-Lodeweyckx 1993, Weltman et al 1994, Daughaday 1995).

HGH release from the liver is balanced between the stimulation by growth hormone releasing hormone (secreted from the nucleus arcuatus) and the inhibition by somatostatin (secreted from the paraventricular nucleus/ proptic nucleus) both released from the hypothalamus and transported to the pituitary gland by the portal circulation. Many stimuli of hGH secretion such as exercise, hypoglycaemia, proteins (arginine) and l-Dopa act through an α-adrenergic mechanism and are inhibited by α-adrenergic receptor blockers such as phentolamine, and are potentiated by the β-adrenergic blocker propranolol. Other neurotransmitters such as CCK, VIP, opioid peptides, γ-aminobutyric acid and acetylcholine, may also modify hGH secretion. However, most of these transmitters seem to act via somatostatin and growth hormone releasing factor rather than directly on the somatotroph cells (Ho et al 1992, Reichlin 1992, Thorner et al 1992, Vance et al 1992, Hartman et al 1993, Parks et al 1993, Cronin and Thorner 1995, Wass and Besser 1995). HGH is shown to increase during exercise already at low intensities and reaches its maximal response at 70 % of maximal oxygen uptake (Galbo et al 1977, Sowers et al 1977, Luger et al 1992). The release of hGH is increased during slow wave sleep, particularly in the beginning of the night, and is inhibited by sleep deprivation (Takahashi et al 1968, Parker et al 1979, Davidson et al 1991, Holl et al 1991, Radomski et al 1992, Pietrowsky et al 1994). Growth hormone releasing hormone promotes sleep in animals (Obal et al 1988, Kerkhofs et al 1993, Krueger and Obal 1993). Whether growth hormone has any sleep promoting effect in humans is, however, debated (Mendelson et al 1980, Åstrøm and Trojaborg 1992, Kern et al 1993).

The dramatic increase seen in the plasma levels of growth hormone during the ranger course, is reversed in the subjects given a high calorie diet. In contrast, 3 hours of sleep each night during the course did not influence plasma levels of hGH. However, the plasma levels of hGH were increased if the blood samples were drawn just after the sleep period (Aakvaag et al 1978, Opstad and Aakvaag 1981, 1983, Paper 1). The absolute hGH response to the bicycle exercise test after an overnight fast was not increased above the enhanced pre-exercise levels during the course (Opstad et al 1980, Paper 1). The high calorie diet prevented the hGH increase during the exercise test during the course. In the control experiment the exercise test was performed after 8 -12 hours of fasting, whereas during the course the subjects of the high energy group could not be kept fasting for more than 4 hours before the exercise test (Paper 1). This shows that nutrients ingested in the

hours preceding the exercise test may abolish the hGH response to an exercise test even during constant strenuous exercise.

Prolactin is one of the most versatile hormones, and its membrane receptors have a wide distribution to very different tissues such as mammary gland, liver, kidney, adrenals, ovaries, uterus, placenta, testis, prostate, seminal vesicles, Leydig cells, hypothalamus, choroid plexus, pancreatic islets, lymphoid tissue, peripheral mononuclear cells, brain, intestine and others. Many tissues are known targets for PRL action, others are not.

Comparative studies indicate that osmoregulation and modulation of growth and development may be the most fundamental actions of prolactin. In humans, functions of PRL are still incompletely delineated but seem mainly to be involved in reproductive functions. PRL increases or maintains the concentration of LH receptors on the Leydig cell membrane, thus increasing the sensitivity of the testis to LH and by that enhancing plasma testosterone levels (Aragona et al 1977). PRL also potentiates the effects of androgens on the growth and secretory activity of male accessory glands and may even have direct androgenic effects. PRL in the seminal fluid also stimulates glucose and fructose utilization and the sperm motility and fertilizing capacity (for reviws see Reichlin 1992, Thorner et al 1992, Cooke 1995).

The pituitary secretion of PRL is under the control of hypothalamic release- and inhibiting factors such as the thyroid-releasing hormone, dopamine, glucocorticoids, oestrogen and epidermal growth factor. The circadian rhythm of PRL, with the highest levels in the early morning hours, may be due to the effect of both sleep and the circadian rhythm of melatonin (Sassin et al 1973, Frantz 1979, Bispink et al 1990, Van Cauter et al 1991, Spiegel et al 1994, Cooke 1995). This nocturnal increase is due to increased amplitude of the secretion pulses which have a frequency of approximately 14 per 24 h (Veldhuis and Johnson 1988). PRL has been shown to increase during different types of stress such as general anaesthesia (halotan), surgery, insulin-induced hypoglycemia and medication with a dopamine blocking effect, such as haloperidol or metoclopramid (Røjdmark and Røssner 1991, Wass and Besser 1995).

During the ranger training course there is a decrease in the plasma levels of PRL which may contribute to the decrease in testicular androgen secretion and which may also contribute to the hypoandrogenisation during the course by more direct ways. The relatively high levels of PRL before the start of the course may indicate that PRL is sensitive to the cadets' anxious anticipation before a strenuous course (Aakvaag et al 1978, Opstad and Aakvaag 1983, Voigt et al 1990, Papers 1 and VI). This is in spite of the fact that academic examination stress did not affect the plasma levels of PRL or hGH (Malarkey et al 1991). No alterations were found during the submaximal exercise test at 50 % of the cadets' maximal oxygen uptake before or during the course. In contrast, others have shown that exercise induces a rise in the plasma levels of PRL (Galbo et al 1977). The explanation is probably that, in contrast to the plasma levels of hGH which increase also at

low exercise intensity, PRL increases only during high intensity exercise, which is 70 % of VO_2 max or more (Luger et al 1992). Since the exercise intensity during the course has a mean of approximately 35 % of VO_2 max, and the bicycle exercise test was 50 % of VO_2 max, this was below the intensity threshold for PRL release during exercise. It is, however, interesting to notice that even the strenuous physical activities during the course did not affect this threshold for PRL release. The small alterations found in the prolactin response to TRH during the course, might indicate that decreased plasma levels of PRL were due to dopamine inhibition, since dopamine is known to inhibit synthesis as well as secretion of PRL.

The adrenals, the gonades and the thyroid gland are regulated by the hypophyseal hormones ACTH, LH/FSH and TSH respectively. LH/FSH decreased during the course and is a main reason for the decreased plasma levels of gonadal androgens. The increased LH/FSH responses to GnRH stimulation during the course may indicate that the decreased gonadotropin levels are due to decreased hypothalamic secretion of GnRH although the decreased levels of androgenic hormones may also contribute to an increased LH/FSH response to GnRH stimulation. GnRH which is detected in septum, preoptic area, amygdala, and midbrain has its highest concentration in the mediance eminence, nucleus arcuatus, and organum vasculosum of the lamina terminalis. Secretion of GnRH is inhibited by dopamine and GABA and stimulated by α-adrenergic agonists, histamine and glutamate. Besides olfactory and visual inputs, GnRH secretion is also influenced by the pineal body and the nucleus suprachiasmaticus. The feedback of gonadal steroids and inhibin both act at the hypothalamic secretion of GnRH and at the pituitary sensitivity to GnRH stimulation (Haug et al 1974, Reichlin 1992, Hall and Crowley 1995, Lincoln 1995, Riskind and Martini 1995, Wasser and Besser 1995, Papers I, IV).

Thyroid hormones are mainly regulated by pitutary TSH which is stimulated by TRH produced in the paraventricular nucleus of the hypothalamus, inhibited by somatostatin produced approximately in the same area, the periventricular area, and by dopamine from the nucleus arcuatus. TRH is also influenced by the thermosensitive cells in the supraoptic nucleus, is stimulated by β-adrenergic agonists and may be inhibited by serotonin. The negative feedback of thyroid hormones acts both at the hypothalamic and at the pituitary level (for review see Reichlin 1992, Scanlon and Hall 1995, Wondisford et al 1995)

The decreased TSH levels during the course would obviously contribute to the decreased thyroid secretion in spite of the fact that there was no direct connection between alterations in thyroid hormones and the decrease in TSH. The TSH response to TRH was reduced by 80 % during the course equally due to the strenuous physical exercise and energy deficiency, whereas sleep had minor significance (Opstad et al 1984).

Adrenal steroids are all stimulated by pituitary ACTH, which is under the control mainly of the hypothalamic CRH, but is also stimulated by AVP (vasopressin). CRH has also been localized to multiple brain areas, the spine and the gastointestinal tract but with the highest

concentration in the hypothalamus, particularly the paraventricular nucleus. The CRH neurones receive excitatory inputs from many brain areas such as nucleus suprachiasmaticus, amygdala and the raphe nuclei and inhibitory inputs from the hippocampus and the locus coeruleus. CRH secretion/release is stimulated both by acethylcholine, serotonin, and interleukin 1 and inhibited by GABA and nor-adrenaline. The negative feedback regulation by glucorticoids may act at the pituitary as well as at the hypothalamic level, but there are also glucocorticoid receptors in various other parts of the brain such as in the amygdala and the hippocampus (for review see Reichelin 1992, Grossman 1995, Imura 1995).

The decreased ACTH levels measured during the training course could be due to the state of recovery just prior to the blood sampling since the subjects had only light physical activities 1-2 hours prior to blood sampling. The short half-life of ACTH compared to the long half-life for cortisol could explain the decreased ACTH levels during recovery (Paper V).

3.7 Mental performance and clinical symptoms

All mental performance and clinical symptoms have a biochemical or physiological basis, the disturbance of which will affect the soldiers' total performance. Sleep deprivation affects a series of mental functions, but does not affect the physical performance to any significant extent (Hill et al 1994, Reilly and Piercy 1994): First all subjective symptoms, such as the subjects' mood state, social well-being, ability to care for others, feeling of depression, and motivation will be affected. Then the most advanced mental performance tasks will be affected such as creativity, ability to solve complex mental performance tasks, or tasks requiring memorization and tactical abilities. With prolonged sleep deprivation the body's requirement for sleep increases so strongly that it becomes impossible to withstand sleep, and subjects will fall asleep even in the upright position. First, however, this will appear during the night-time, particularly in a period with rather low physical activities, in the form of extreme tiredness followed by balance disturbance, problems with straight walking along the roads, later with pseudo- or real illusions and hallucinations. In the beginning of the course the subjects take the hallucinations for real signs, whereas at the end of the course when they have got more used to them, they most often take all unexpected events as hallucinations. This might be a serious impairment of their function because they are not able to differentiate between real and not real signs and will be completely inadequate for watchkeeping or surveillance tasks. These periods with intense feeling of sleepiness induce what has been called "microsleep" or lapses (Walter Reed Laps Hypothesis). The periods of microsleep increase in frequency and length almost proportionally to the length of sleep deprivation. The length might be from some seconds to several minutes and the frequency from some times a day to several times each hour, but still depending on the length of sleep deprivation and time of day (Johnson and Naitoh 1974, Opstad et al 1978, Haslam 1983, 1984, Angus et al 1987). During these periods the

vigilance is so reduced that the subjects do not record even distinct signals in the environment. In the so called vigilance tests this gives error of omissions. In contrast, there are not many faults or wrong responses. The reaction time also increases and consequently all tasks therefore take more time than normal. On the third night of total sleep deprivation, the more serious signs appear, such as slow motions, balance disturbance, nystagmus, fog sight, disturbed distance vision, headache, hallucinations and physical exhaustion. In this state the soldiers are helpless and unable to manage their own situation. In stead of being a resource for the platoon, they become a problem. In such a state it is often observed that the cadets only disappear from the platoon or just sit/lay down and may fall into a state of sleep narcosis. They may be extremely hard to wake up. Due to the internal sleep rhythm of approximately 90 minutes they may be much easier to wake up after only a few minutes. However, a sleep deprived person is possible to wake up for some seconds and then he will normally be oriented for time and site. This will make the difference to unconsciousness due to commotio cerebri which will be impossible to wake up by external stimulation.

Mental performance has a circadian rhythm with the highest performance in the afternoon and the lowest performance at night (±10-15%) (Bugge et al 1979, Czeisler et al 1980, Nickolson and Stone 1982, Folkard et al 1985). During the course there is a general decrease in mental performance; in addition the decrease is stronger during night-time than during day time leading to an increased amplitude of mental performance. This is not only due to the darkness of the night but also to endogenous circadian rhythms, since the same alterations are found in the courses organized in June, when the nights are rather light. All clinical symptoms are also more pronounced at night than at day-time. Almost all illusions, misperceptions, hallucinations, balance disturbances, and coordination problems are worse at night than during the day.

In contrast to the alterations in the circadian rhythm for mental performance which show increased amplitude during the course, the circadian rhythm of hormones are all extinguished during the course. The circadian rhythms are regulated from the nucleus suprachiasmaticus in the anterior hypothalamus (Van den Pol and Powley 1979, Rietveld 1992). This center is thought to regulate most of the known circadian rhythms. Previously it has been shown by Folkard (1985) that the period of the circadian rhythm for the feeling of alertness or drowsiness and deep body temperature dissociated when the period was shortened by 0.2 hours each day down to a "day" of 23 hours and then by 0.1 hour until a "day" of 22 hours which was run for the rest of the study. During the present military training course, it is shown for the first time that during continuous operations, a dissociation may appear between the amplitude of the circadian rhythm for mental performance which is increased, and the amplitude of circadian rhythm for steroid hormones which is extinguished. This indicates that these two rhythms are regulated by different mechanisms in the brain (Paper VII).

3.8 Conclusions

The present investigation shows rather large endocrine and metabolic alterations during a 5 day military training course with continuous physical activities combined with sleep and energy deficiency which might contribute to explain the accompanying alterations in both mental and physical performance. The main stress factors such as physical strain, lack of food and sleep all lead to an extreme catabolic metabolism with high priority for energy production.

One main finding is an adrenergic desensitization, in that the catecholamine responses to a standardized exercise test were strongly increased in spite of minor alterations in pulse rate and blood pressure during the same exercise test. This desensitization was mainly due to the physical strain and is explained both by a decreased number and sensitivity of the adrenergic receptors and a decreased cAMP response to adrenaline stimulation. These alterations indicate that an important mechanism for reduced physical performance or exhaustion is target tissue desensitization.

The increased plasma levels of catecholamines during the course were mainly due to the effect of physical exercise, since extra food or sleep did not reverse these alterations. In contrast the increased cortisol and growth hormone responses to exercise were partly reversed by extra food, whereas extra sleep did not have any major influence.

Conjugated catecholamines increased during prolonged exercise but only marginally during short term exercise. Conjugated catecholamines did not serve as a source for free catecholamines during exercise and played a minor role for their inactivation post exercise. The great interindividual variations in plasma conjugated dopamine were due to food dopamine, since they disappeared during the course.

The decreased levels of adrenal and testicular androgens and thyroid hormones may also contribute to alterations in both mental and physical performance. In addition the decreased thyroid hormones during the course mainly due to energy deficiency, may affect the cadets' cold tolerance. There is a dissociation between the circadian rhythm for mental performance, which is increased, and the circadian rhythms for steroid hormones, which are extinguished. The abolished night time secretion of steroid hormones indicates that night activities are particularly strenuous and demanding for the cadets.

Some of these alterations are advantageous or even a necessary adaptation in order to maintain mental and physical performance capacity, whereas other alterations may even be harmful and produce disease and should be avoided or treated. Some simple precautions or countermeasures, such as adjustment of the type and amount of food and sleep, may be sufficient to preserve the soldiers' mental and physical performance and prevent unnecessary health hazards.

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Paper I: Legends to figures 2 and 3 have been exchanged.

PAPER I

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Alterations in the morning plasma levels of hormones and the endocrine responses to bicycle exercise during prolonged strain. The significance of energy and sleep deprivation

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Abstract. The relative significance of physical exercise, energy and sleep deprivation for the morning levels of hormones and the endocrine response to short-term bicycle exercise were investigated in 24 male cadets during a 5-day military training course. Significant increases in the morning levels of noradrenaline, adrenaline, and dopamine, and a decrease in PRL were ascribed mainly to physical strain. Cortisol and hGH increased, whereas insulin and glucose decreased mainly due to energy deficiency. Pulse rate after the bicycle test was unchanged and similar in all groups in spite of increased catecholamine responses. The increased catecholamine response was mainly due to physical strain. The cortisol response to the bicycle test was increased in all groups, and energy deficiency caused slower postexercise recovery. The incremental hGH response to the exercise test was unchanged in the energy-deficient subjects but abolished in the well-fed subjects. The results suggest that the endocrine responses during long-lasting exhausting strain were mainly due to physical exertion and energy deficiency, whereas sleep deprivation did not play any major role.

Physical exercise is known to elicit a multitude of endocrine and metabolic responses appropriate for energy mobilization and cardiovascular adaptation. Thus, there is a gradual increase in the plasma levels of noradrenaline, adrenaline, dopamine and pulse rate with increasing exercise intensity (1,2). The serum levels of cortisol and growth hormone are known to increase when a certain workload is exceeded (3-6). Short-term physical exercise has variable effects on serum prolactin (7-10), and a decrease has been shown during long-term exercise (11). Serum insulin decreases during short-term exercise, and glucose is slightly increased (10,12).

Only moderate peripheral endocrine and metabolic alterations have been found during sleep deprivation (13). Cortisol, hGH and PRL have been shown to increase during night-time, but the distinction between the effect of sleep and the natural circadian rhythm is difficult. It seems, however, as if sleep as such increases the serum levels of hGH and cortisol (13,14).

In contrast to sleep deprivation, energy deficiency causes profound changes in the metabolic and endocrine state of the human body (15-17). There are alterations both in the secretion and degradation of hormones and metabolites. The action of hormones on target tissues is altered as are the energy stores. hGH and cortisol have been shown to increase during starvation, whereas the thyroid function is decreased (18,19).

Previously, considerable endocrine and metabolic alterations have been documented during a 5-day military training course with heavy physical exercise, sleep and energy deficiency (10-12,14,20,21).

Some of these results were found to be dependent on sleep and/or nutritional factors as studied in separate courses where the cadets were given either extra amounts of sleep or additional food supplies. In these courses, blood samples were drawn immediately after the sleep period, which

may have resulted in the detection of hormonal changes induced by sleep itself.

In the present investigation the effects of extra food or 3 hours additional sleep on an extended number of endocrine responses were compared in the same experiment. Immediate effects of sleep were avoided by organizing the extra sleep period 6-8 hours before blood sampling. Alterations in the morning levels of hormones during the training course were measured. In addition, one main object of the study was to determine the significance of nutrition and extra sleep for the endocrine responses to a standardized exercise test after prolonged exhausting stress.

Subjects and Methods

Training course

The subjects were 24 first year cadets of the Norwegian Military Academy who participated in a ranger training course for 5 days as a part of their school programme. The subjects were randomly divided into three groups. Group 1 consisted of 9 subjects with a mean age of 22 years (range 21-25). They were exposed to continuous physical exercise (35% of maximal oxygen uptake as a mean) representing a daily energy consumption of about 35 000-45 000 kJ. The subjects did not get any scheduled sleep during the course. By observation and heart rate recordings we found that they slept for a total of 1-3 h during the entire course. Group 2 consisted of 7 cadets with a mean age of 24 years (range 22-27) who differed from Group 1 in that they received a specially composed diet. Group 3, consisting of 8 cadets with a mean age of 23 years (range 22-25), had 3 h of sleep each night, usually between 21.00 and 03.00 h. While this group slept, the others had activities only enough to keep them awake.

Diet

The daily basic food intake for all the cadets consisted of approximately 60 g of proteins, 40 g of fat, 100 g of carbohydrates, and 1-2 g of NaCl, representing about 4000-5000 kJ/24 h. Group 2 was in addition given a specially composed diet representing about 30 000-35 000 kJ/24 h for each cadet. This diet contained 100 g of proteins, 130 g of fat, 1400 g of carbohydrates, and 20-30 g of NaCl. The extra diet was given as soup, orange juice, cocoa, and milk shake. The high carbohydrate content was achieved by adding a preparation easily soluble in water, nearly tasteless and colourless (maltodextrin). Because of this special diet the subjects of Group 2 consumed about 6 l liquid each day. The cadets were drinking water and liquid from their canteens, and reported the amount of liquid consumed. This amount was roughly the same in all groups. Because of the high energy diet, Group 2 did not have any significant loss of weight during the course. In Groups 1 and 3 with an energy deficit of 30 000-40 000 kJ/24 h, each cadet had a weight loss of about 4 kg, mostly fat.

Blood sampling

All tests and blood sampling were performed between 06.00 and 09.00 h daily during the course. All blood samples were obtained from a Veneflon® cannula in the antecubital vein, which was inserted 5-10 min before blood sampling. The amount of blood taken each time was about 40 ml. Blood for preparation of plasma was centrifuged immediately in a refrigerated centrifuge, whereas blood for the preparation of serum was allowed to clot for about 30 min, and then centrifuged in a refrigerated centrifuge. Aliquots of serum and plasma were frozen immediately on dry ice and kept frozen at −80°C.

Bicycle exercise

The maximal oxygen uptake (VO_2 max) was estimated for each cadet before the course by measuring the heart rate at three separate submaximal workloads on a bicycle ergometer. The mean value of estimated VO_2 max from the Aastrand & Rhynning nomogram was used. On day 3 during the course and in a control experiment some months after the course, the cadets were tested by 15 min ergometer bicycle exercise of approximately 60% of maximal oxygen uptake (workload 100-130 W). The control experiment was performed indoors with a room temperature of about 21°C. During the course, the exercise test was performed outdoors in the training area at about 500 m altitude. The weather was fairly good with a temperature of about 20°C. Blood samples were drawn just before the bicycle exercise with the subjects sitting on their bicycles, at the end of the 15 min of exercise, and with the subjects recumbent in their sleeping bags after 15 and 25 minutes of recovery.

TRH test

The TRH test was performed on day 1 just before the start of the course and on day 4 during the course, both days between 06.00 and 08.00 h in the morning with the subjects recumbent in sleeping bags. Blood samples were taken just before and furthermore 30 and 60 min after the iv injection of 0.4 mg TRH (Hoechst, FRG).

Chemical analysis

The catecholamines were analysed with a radioenzymatic method (22). Radioimmunoassays were used for the other hormone analyses. Cortisol was determined with the GammaCoatKit from Clinical Assays, (MA, USA). hGH, insulin and prolactin were measured with kits from Immuno Nuclear Co (MN, USA). Glucose was analysed using the hexokinase method (Boehringer, FRG).

Statistics
The results are presented as means ±SEM. An analysis of variance for repeated measures was used to test alterations within the same group and between different groups (Manova, SPSS). The t-test was used to identify the significant differences and day-to-day differences. Pearson's correlation coefficient was calculated using SPSS. Non-parametric statistics were applied to the growth hormone results, since these data did not show normal distribution; Friedman's two-way ANOVA to test alterations within the same group and Kruskal-Wallis' one-way ANOVA to test differences between the different groups (SPSS). Wilcoxon rank-sum and sign test were used to identify significant differences.

Results

Plasma noradrenaline levels (nmol/l) increased about 10-fold during the course, from 1.1±0.2 to 11.4±1.2, from 1.1±0.1 to 12.0±1.4, and from 0.9±0.2 to 10.5±1.2 in Groups 1, 2 and 3, respectively ($F_{6,126}$=112.4, p<0.0005) (Fig. 1). Using an overall analysis of variance for repeated measures (Manova, SPSS) Group 2 was significantly ($F_{12,126}$=3.17, p=0.001) different from Groups 1 and 3, which did not differ. The t-test showed significantly lower levels for Group 2 only on day 4 (T_{22}=2.28, p=0.033). The absolute noradrenaline response to the bicycle test was increased in all subjects during the course (Fig. 2) ($F_{3,63}$=8.85, p<0.0005), whereas the relative response was unchanged. The noradrenaline response during the exercise test ($F_{3,63}$=83.87, p<0.0005) was not significantly different between the three groups, either in the control experiment or during the course. The noradrenaline concentrations were unaffected by TRH iv (Table 1).

Plasma adrenaline (nmol/l) (Fig. 1) increased 3-5 fold during the course from 0.33±0.04 to 1.31±0.19 in Group 1, from 0.30±0.04 to 1.31±0.27 in Group 2, and from 0.18±0.04 to 1.40±0.19 in Group 3 ($F_{6,126}$=33.4, p<0.0005). There were no significant differences between the three groups. The adrenaline response to the bicycle test ($F_{3,63}$=55.8, p<0.0005) was increased during the course ($F_{3,63}$=3.8, p=0.014) (Fig. 2), without significant differences between the three groups. Plasma adrenaline was not influenced by TRH stimulation iv (Table 1).

Plasma dopamine concentrations (nmol/l) (Fig. 1) increased from 0.16±0.04 to 1.03±0.12 in Group 1, from 0.18±0.05 to 1.01±0.21 in Group 2, and from 0.18±0.05 to 1.29±0.15 in Group 3 ($F_{6,126}$=46.83, p<0.005). Significantly lower levels were found for Group 2 compared with the others ($F_{12,126}$=2.48, p=0.006) only on day 4 (T_{22}=2.79, p=0.011). A small but significant increase ($F_{3,63}$=13.18, p<0.005) was also seen during the exercise test, without significant differences between the three groups (Fig. 2). TRH stimulation iv did not affect the plasma concentrations of dopamine.

Pulse rate (beats/min) (Fig. 2) was increased in all three groups during the course ($F_{3,63}$=9.79, p<0.0005), whereas the same pulse rate was reached after 15 min of exercise ($F_{3,63}$=728.98, p=<0.0005) both during the course and in the control experiment. There were no significant differences between the three groups.

Serum cortisol concentration (nmol/l) (Fig. 3) increased about 2-fold during the course ($F_{6,90}$=13.40, p<0.0005), from 400±31 to 787±70

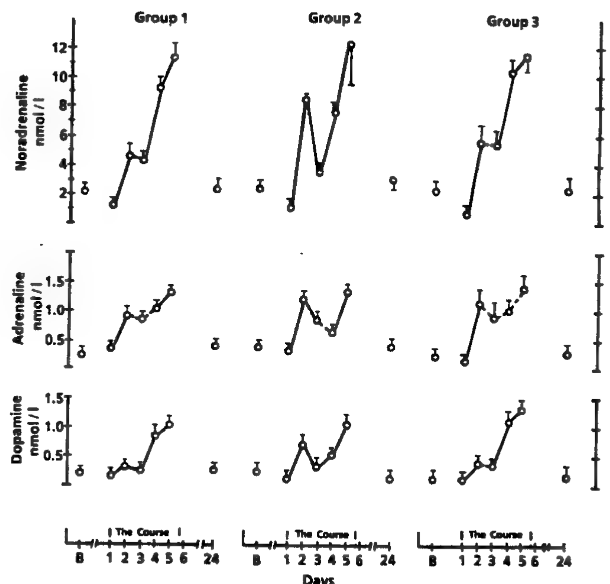

Fig. 1.
Alterations in the morning levels of noradrenaline, adrenaline and dopamine during a 5-day military training course. The basal (B) values were obtained two weeks before the course. Another baseline was obtained after 24 days of recovery with ordinary school activities. The subjects in Group 1 (N=9) were exposed to continuous physical activities combined with energy and sleep deprivation. The subjects in Group 2 (N=7) were compensated for the energy deficiency, and Group 3 (N=8) was given 3 h of scheduled sleep each night. The day-to-day variations significant at p<0.01 are shown by thick lines, and variations not significant are shown by dotted lines. The values are given as means ±SEM.

in Group 1, and from 392±36 to 850±75 in Group 3. A small but significant increase was found during the course in Group 2 ($F_{6,36}=2.47$, $p<0.042$) which had significantly lower levels throughout the course than Groups 1 and 3 ($F_{12,126}=3.1$, $p=0.001$). During the bicycle exercise test, a significant decrease was found for plasma cortisol in the control experiment ($F_{3,63}=4.86$, $p=0.004$), whereas a significant increase ($F_{3,63}=10.52$, $p<0.0005$) was found during the course in all groups (Fig. 4). In the energy-deficient groups the increase continued for 10 min into the recovery phase, and levels had not fallen to pre-exercise values even 25 min after exercise

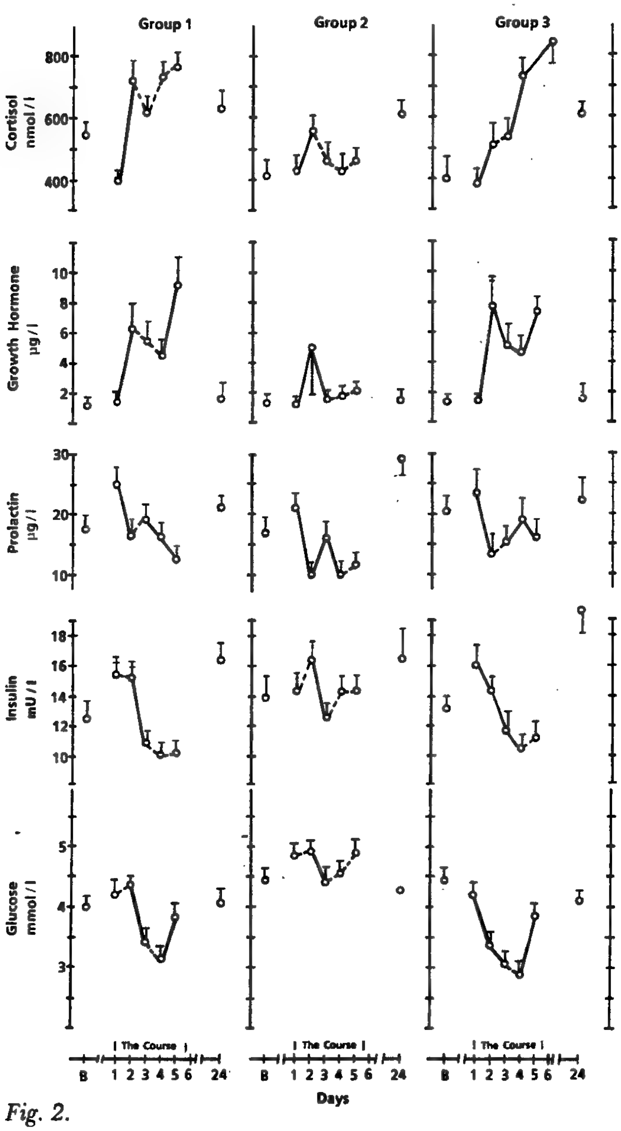

Fig. 2.
Noradrenaline, adrenaline, dopamine, and pulse rate during bicycle exercise with 60% of VO_2 max (0-15 min) and during the following recovery (15-40 min). The bicycle experiment was performed after prolonged strain (Δ–Δ) and in a control experiment (O–O) some months after the course. For details, see Fig. 1.

($F_{6,63}=2.36$, $p=0.041$). In Group 2, the cortisol level decreased gradually during the recovery period and pre-exercise levels were obtained after 25 min of recovery.

Human growth hormone (Fig. 3) concentrations (µg/l) increased 5-10 fold during the course, from 1.7±0.4 to 9.1±2.0 in Group 1 (CS(chi square)$_6$=30.0, $p<0.00005$), and from 1.6±0.2 to 7.2±0.9 in Group 3 ($CS_6=33.58$, $p<0.0005$). A small but significant increase was also found in Group 2 ($CS_6=15.95$, $p=0.014$). No significant difference was found between Group 1 and 3. Group 2 had significantly lower plasma levels of growth hormone during the course than Groups 1 and 3 when comparing integrated values by Kruskal-Wallis' one-way ANOVA ($CS=7.73$, $p=0.0209$). Differences between the groups for each day were: day 2: $CS=6.39$, $p=0.041$; day 3: $CS=11.03$, $p=0.004$; day 4: $CS=8.83$, $p=0.0121$; day 5: $CS=12.06$, $p=0.0024$. In spite of higher basal levels, the incremental hGH response to the exercise test was not altered in Groups 1 and 3 during the course (Fig. 4). The response in Group 2 was completely abolished. hGH in serum was not affected by TRH iv (Table 1).

Serum prolactin concentrations (µg/l) (Fig. 3) decreased during the course, from 25.4±3.6 to 12.5±1.8 in Group 1, from 21.8±2.7 to 10.1±1.5 in Group 2, and from 23.0±4.0 to 13.1±2.2 in Group 3 ($F_{6,126}=16.93$, $p<0.0005$). The subjects in Group 2 had significantly lower levels than the others ($F_{12,126}=2.84$, $p=0.002$). No significant alterations were found during the bicycle test (Fig. 4). The PRL response to TRH stimulation ($F_{2,16}=65.44$, $p<0.0005$) was significantly increased during the course in Group 1 ($F_{2,16}=6.45$, $p<0.009$). No significant alterations were found in the PRL response to TRH for Groups 2 and 3 during the course (Table 1).

Serum insulin* levels (mU/l) (Fig. 3) decreased during the course in the energy-deficient groups ($F_{6,84}=18.42$, $p<0.0005$), from 15.7±1.3 to 10.1±0.5 in Group 1, and from 16.0±1.6 to 10.5±1.0 in Group 3, whereas no significant alterations were seen in Group 2. During the exercise test (Fig. 4) the insulin levels decreased, with a subsequent increase after 10 min of recovery ($F_{3,60}=36.51$, $p<0.0005$). Pre-exercise levels were obtained after 25 min of recovery. The same response pattern was found during the course, but at

significantly lower levels ($F_{1,20}$=17.89, p<0.0005). Serum insulin levels were not affected by TRH iv.

Serum glucose concentration (mmol/l) (Fig. 3) decreased during the course in the energy-deprived subjects ($F_{6,90}$=16.80, p<0.0005), from 4.2±0.3 to 3.2±0.2 in Group 1 and from 4.2±0.1 to 2.9±0.1 in Group 3, whereas no significant alterations were found in subjects receiving the high-calorie diet. During the bicycle test (Fig. 4) no significant alterations were seen in serum glucose concentrations in the control experiment or during the course in the subjects receiving the high-calorie diet. In contrast, a significant decrease was found in the energy-deficient subjects during the course ($F_{3,45}$=12.87, p<0.0005) (Fig. 4).

Correlations

During the course, significant correlations were found between the noradrenaline and adrenaline response on day 2 (R=0.70, p=0.0005), day 3 (R=0.49, p=0.007), day 4 (R=0.50, p=0.007), and day 5 (R=0.58, p=0.001). Significant correlations were also found between the alterations in noradrenaline and dopamine on day 2 (R=0.47, p=0.010), day 4 (R=0.61, p=0.001), and day 5 (R=0.52, p=0.005), and between adrenaline and dopamine on day 4 (R=0.61, p=0.001) and day 5 (R=0.52, p=0.005).

Discussion

This study shows that during prolonged multifactorial strain, there is an increase in the plasma levels of catecholamines and a decrease in prolactin mainly owing to physical strain. Cortisol and growth hormone increased, and glucose and insulin decreased mainly owing to energy deficiency. The study further shows that sleep deprivation seems to play only a minor role in these alterations, when blood sampling takes place several hours after the sleep period. During a previous course, when the blood samples were drawn just after the

Table 1.
The hormonal response to TRH (0.4 mg iv) stimulation before and after 4 days of heavy physical activity combined with energy and sleep deprivation. Blood samples were drawn just before, 30 and 60 min after TRH injection. The results are presented as means ± SEM

	Group	Control			Stress		
		0 min	30 min	60 min	0 min	30 min	60 min
Prolactin μg/l	1.	24.0±3.2	57.3±6.8	39.6±4.8	14.7±2.0	66.6±6.0	40.7±3.7
	2.	21.8±2.7	74.0±17.0	45.7±8.5	10.5±1.7	64.9±1.3	43.4±8.0
	3.	22.1±4.1	48.4±7.2	32.5±5.0	20.1±2.6	41.6±5.6	33.0±4.8
Growth hormone μg/l	1.	1.7±0.4	2.0±0.4	2.2±0.6	4.4±0.9	4.2±0.8	3.7±0.8
	2.	1.2±0.2	2.3±0.5	2.6±1.0	1.9±0.4	1.3±0.2	1.5±0.3
	3.	1.6±0.2	1.9±0.4	1.8±0.2	4.7±0.7	4.2±1.1	4.2±1.1
Insulin mU/l	1.	14.7±1.6	12.6±0.7	14.0±0.9	10.1±0.5	9.7±0.4	10.4±0.9
	2.	14.4±1.2	13.7±1.0	14.4±1.9	14.4±1.1	12.4±1.4	13.3±1.1
	3.	16.1±1.6	14.3±0.8	15.2±0.8	10.5±1.0	10.0±1.0	10.8±0.9
Noradrenaline nmol/l	1.	1.1±0.2	0.9±0.1	1.0±0.2	9.1±0.9	6.9±0.8	7.5±1.1
	2.	1.1±0.1	1.3±0.2	1.2±0.1	6.7±0.7	6.9±0.9	7.7±0.6
	3.	1.0±0.2	1.0±0.2	0.9±0.2	9.6±1.0	8.6±1.5	8.6±1.6
Adrenaline nmol/l	1.	0.33±0.04	0.33±0.05	0.30±0.05	1.02±0.21	0.91±0.24	0.98±0.31
	2.	0.30±0.04	0.42±0.08	0.34±0.08	0.65±0.18	0.65±0.22	0.68±0.31
	3.	0.18±0.02	0.19±0.05	0.17±0.02	1.01±0.19	1.06±0.03	0.80±0.14
Dopamine nmol/l	1.	0.16±0.04	0.16±0.03	0.18±0.05	0.89±0.13	0.66±0.11	0.78±0.09
	2.	0.18±0.05	0.18±0.08	0.19±0.09	0.50±0.14	0.75±0.25	0.78±0.22
	3.	0.18±0.05	0.26±0.10	0.21±0.06	1.14±0.13	1.12±0.15	1.02±0.17

sleep period, significantly higher levels were found for plasma cortisol, hGH and testosterone, indicating that sleep itself might stimulate the release of these hormones (14).

During the present course, 3 hours of sleep each night was chosen because it was the amount allowed by the Military Academy in order not to hamper the military training programme. However, even though 3 hours of sleep each night is still sleep deprivation, the most serious deterioration in behaviour, performance and clinical symptoms appears when sleep is reduced from 3 hours each night to no sleep at all. Almost all cadets totally sleep-deprived are hallucinated during the course. In contrast, hallucinations are rare in subjects allowed 3 hours of sleep each night (23). The most profound endocrine alterations owing to sleep deprivation should therefore be expected when the amount of sleep is reduced to below 3 hours/24 hours.

Previously we have shown that the catecholamine response to the bicycle exercise test was strongly increased after prolonged stress (10). The present investigation with identical workload in the control experiment and the stress experiment shows that this increased response was mainly due to preceding physical strain.

In the rat it has been shown that fasting reduces noradrenaline turnover in most tissues except the liver (17,24,25). Fasting in humans has been shown to decrease urinary excretion of noradrenaline and increase the urinary excretion of adrenaline (24).

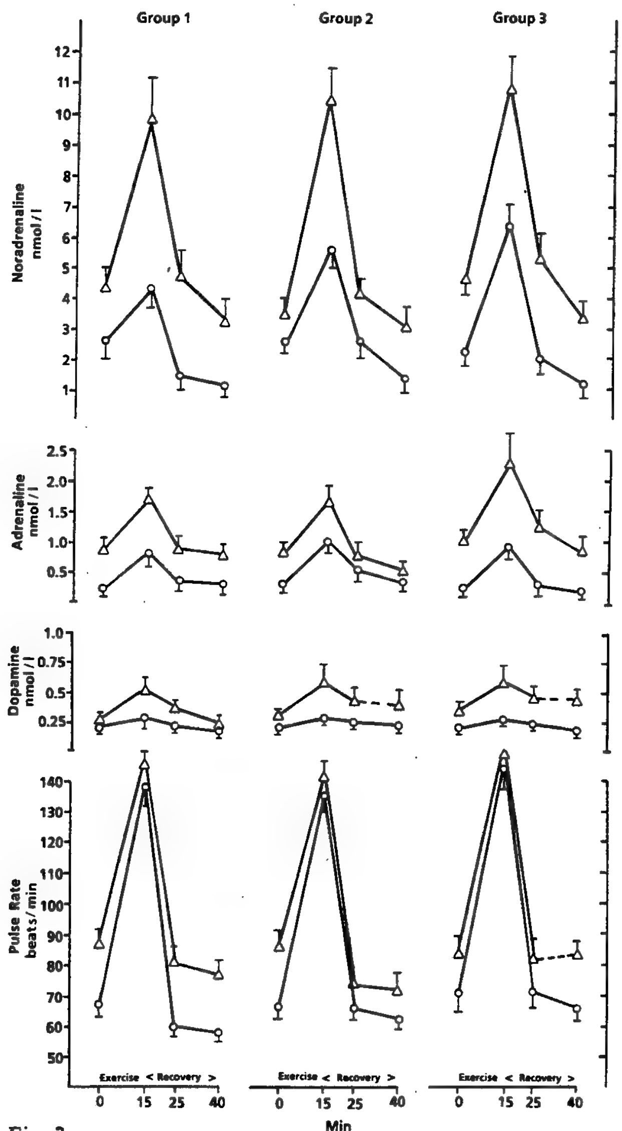

Fig. 3.
Alterations in the morning levels of cortisol, hGH, PRL, insulin, and glucose during a 5-day military training course. For details, see Fig. 1.

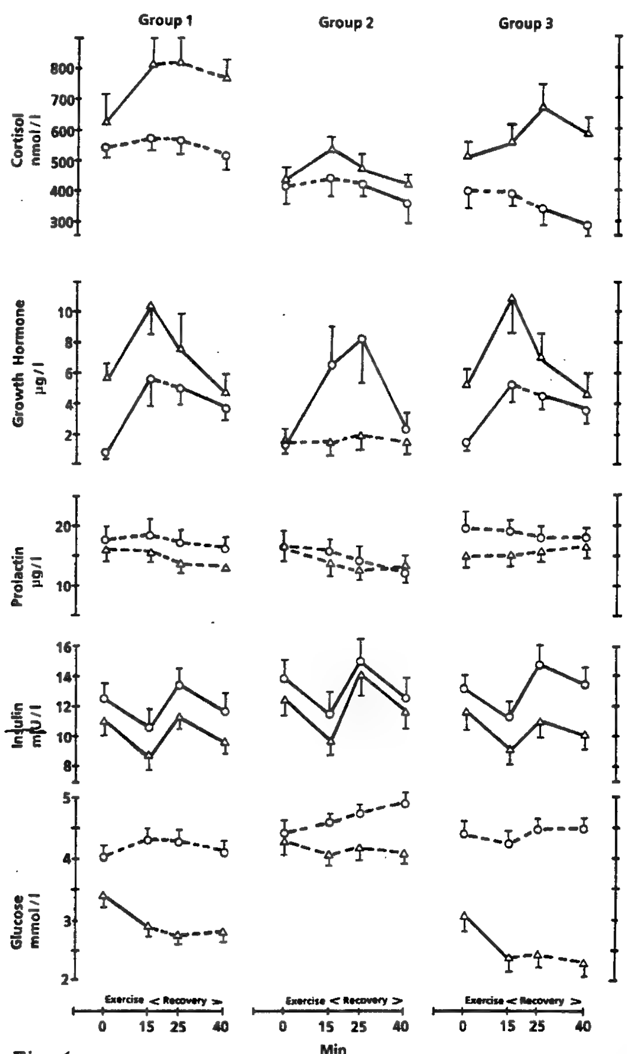

Fig. 4.
Cortisol, hGH, PRL, insulin and glucose responses to the exercise test before and after prolonged strain. For details, see Figs. 1 and 2.

Conversely, refeeding increases the urinary excretion of noradrenaline and reduces the excretion of adrenaline. These results indicate a suppression of sympathetic activity and stimulation of the adrenal medullary activity during fasting. On the other hand, others have shown that fasting increases the basal plasma levels of noradrenaline but does not change the basal plasma levels of adrenaline, whereas the noradrenaline and adrenaline response to bicycle exercise both are increased (8,26). On this background it is surprising that there is no significant difference between the energy-deficient and the well-fed subjects during the present course. A possible explanation is that all the subjects were in a state of starvation after 6-8 hours without food, and undergoing strenuous activities. Another explanation is a metabolic adaptation to prolonged physical strain. The increased catecholamine levels during the course and the increased response to the exercise test in all groups show that the effect of prolonged strenuous exercise dominates over the effect of energy deficiency.

Even if plasma noradrenaline, adrenaline and dopamine often respond to the same environmental factors, exercise mostly induces noradrenaline secretion, whereas hypoglycemia elicits adrenaline release. The role for dopamine is not so well established. The significant correlation between the different catecholamines indicates a 20-30% common activation of the three catecholamines during prolonged physical stress.

It has been shown that plasma catecholamines are increased in salt-deficient subjects (27,28). The subjects in Groups 1 and 3 got only 1-2 g of salt each day, whereas the subjects in Group 2 had 20-30 g of salt each day in their diets. It is therefore noteworthy that this difference in salt intake was not reflected in the plasma catecholamine levels.

In spite of the increased catecholamine response to the bicycle test there was no corresponding increase in the pulse rate response, indicating an adrenergic desensitization. This desensitization during the course may be due to a downregulation of the adrenergic receptors. Other explanations might be that the plasma levels of noradrenaline do not reflect the release of noradrenaline in the sympathetic nerve terminals in the heart, or that there is a concomitant activation of the parasympathetic nervous system, which counterbalances the increased sympathetic activation.

In accordance with previous investigations (5,6,10), cortisol showed a slight decrease during 15 min of bicycle exercise with 60% of VO_2 max in the control experiment. Cortisol is known to promote gluconeogenesis, and the increased response to the bicycle test during the course may indicate that the subjects activated the gluconeogenesis earlier during the course than in the control experiment. Even in subjects in approximate energy balance the plasma cortisol increased during the exercise test after prolonged strain. The main consequence of energy deficiency on the cortisol level is the slower postexercise recovery.

Growth hormone is known to increase during exercise, and has glucose-sparing effects (10,29,30). The incremental hGH response to short-term bicycle exercise was, however, not increased during the course despite increased basal levels. The abolished hGH response to the bicycle test in the subjects receiving the high-energy diet is in accordance with results by others (31-33) who have shown that the hGH response to exercise is reduced by prior glucose injection, whereas the cortisol response is less affected.

The decrease in serum prolactin during the course was mainly due to physical exercise with a very small contribution from energy deficiency, whereas sleep deprivation did not have any significant influence. PRL did not change during short-term bicycle exercise in contrast to the findings by Sowers et al. (7) and Galbo et al. (31).

The fact that the PRL response to TRH was slightly increased during the course in the subjects in Group 1, and not in those in Groups 2 and 3, indicates a small effect of sleep and energy deprivation on the PRL response to TRH. This is in accordance with observations by Carlson et al. (34), but in contrast to the blunted PRL response to TRH after 36 hours of fasting found by Vinik et al. (35).

Other hormones investigated were not influenced by TRH stimulation. The increased noradrenaline concentrations found by Morley et al. (36) may be explained by the sensitivity of plasma catecholamines to all sorts of environmental and experimental conditions.

As expected, plasma glucose decreased during the course, and during the bicycle exercise test only in the energy-deficient subjects. In the control experiment, insulin decreased during the exercise test followed by an increase 10 min post exercise (10). The same response pattern was observed during the course as in the control experiment, although at significantly lower levels.

In conclusion, this investigation shows that the large endocrine and metabolic alterations found during prolonged strain combined with sleep and energy deficiency were due to a combination of physical exercise and energy deficiency, whereas sleep deprivation was of only minor significance.

Acknowledgments

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PAPER II

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ADRENERGIC DESENSITIZATION AND ALTERATIONS IN FREE AND CONJUGATED CATECHOLAMINES DURING PROLONGED STRAIN, SLEEP AND ENERGY DEFICIENCY

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The pulse rate, blood pressure, free and conjugated catecholamine response to a bicycle exercise test with a workload of about 60% of VO_2max were investigated in a control experiment and after 4–5 days of continuous exercise combined with sleep and energy deficiency. In a separate group the effect of glucose infusion during the exercise test was investigated. The free catecholamine response during the exercise test was increased during the course with only small variations in the pulse rate and blood pressure responses. Glucose infusion during the exercise test markedly reduced the noradrenaline response, while the pulse rate response was only slightly affected. Conjugated noradrenaline and adrenaline increased 2–3-fold during the course, whereas conjugated dopamine did not change. There were only small variations in the plasma levels of conjugated catecholamines during the 30-min bicycle exercise test. Great inter-individual variations in conjugated dopamine levels were found in the control experiment, performed after an overnight fast. These variations were reduced during the course, indicating that they could be caused by environmental factors such as nutrients.

Key words: stress; sleep-deprivation; energy deficiency; exercise; catecholamines; blood pressure; pulse rate.

INTRODUCTION

Catecholamines, important regulators of homeostasis, are indispensable in the physiological adaptation to different environmental conditions and demands such as exercise, cold, fasting, mental stress, surgical operations and diseases (Unger *et al.*, 1980; Dunne *et al.*, 1984; deChamplain *et al.*, 1984; Peyrin *et al.*, 1986; Kuchel *et al.*, 1986; Ratge *et al.*, 1986; Pluto *et al.*, 1987)

The adrenal medulla is the source of all plasma adrenaline, whereas noradrenaline has a dual origin, the sympathetic nerve terminals and the adrenal medulla. The main source of plasma noradrenaline is the nerve terminals in the sympathetic nervous system. Plasma noradrenaline is therefore thought to reflect the degree of activity in the sympathetic nervous system. Plasma dopamine derives mainly from sympathetic ganglion interneurones where dopamine is assumed to be a transmitter. There is also a net release of dopamine from the adrenal medulla (Unger *et al.*, 1980; Lackovic and Neff, 1983; Yoneda *et al.*, 1984, 1985; Scheurink *et al.*, 1989). Peripheral catecholamines are not of central origin, since these substances do not cross the blood–brain barrier (Kuchel *et al.*, 1985).

The degradation or inactivation of plasma catecholamines is by enzymatic *O*-methylation (by the cytoplasmic enzyme catechol-*O*-methyl transferase) to methylated amines such as metanephrine, normetanephrine and methoxythyramine, or by

oxidation by the mitochondrial enzyme monoamine oxidase to the acetic compound, or both, to give vanyl mandelic acid or homovanyl mandelic acid. These inactivations are irreversible, whereas the sulfoconjugation is to some extent reversible (Peyrin *et al.*, 1986). It is still an open question to what extent this might have physiological significance. However, 80% of circulating catecholamines are in the conjugated form, for humans mostly sulfoconjugated. This is particularly important for dopamine which for more than 95% circulates in the conjugated form. The sulfated amines are thought to be biologically inactive. It has been shown, however, that sulfated noradrenaline may bind to the α-receptors but not to the β-receptors and that sulfated dopamine may inhibit the adrenocorticotropic or angiotensin II stimulated aldosterone secretion, (Buu and Kuchel, 1979; Buu *et al.*, 1981; Alexander *et al.*, 1984; Kyncl *et al.*, 1985; Yoneda *et al.*, 1985).

Infusion of free catecholamines results in an immediate increase in conjugated dopamine, whereas the conjugation of noradrenaline and adrenaline is slower due to different affinity for the phenol-sulfo-transferase (Kuchel *et al.*, 1986).

Some investigations have observed an inverse relationship between free and conjugated catecholamines in plasma (Vandongen, 1984; Kuchel and Buu, 1985). This has promoted the discussion whether the sulfated catecholamines might serve as a reserve pool in order to maintain stable levels of free catecholamines. There are, however, conflicting results whether the conjugated catecholamines are unchanged, increased or decreased after physical exercise (Joyce *et al.*, 1982; Cleroux *et al.*, 1983; Davidson *et al.*, 1984; Dunne *et al.*, 1984; Vandongen, 1984; Sothmann *et al.*, 1987).

Previously we have shown that the plasma catecholamine response to a bicycle exercise test was strongly increased in military cadets exposed to 4–5 days of heavy physical activities, sleep and energy deficiency. There was no similar increase in the pulse rate response, indicating a peripheral adrenergic desensitization. This is in accordance with the decrease found in number and affinity of the β_2-receptors on granulocytes and mononuclear cells (Opstad *et al.*, 1980; Opstad, 1990; Opstad *et al.*, 1990).

During the present course we also wanted to study the influence of prolonged physical strain, sleep and energy deficiency on blood pressure, and plasma conjugated catecholamine responses to astandardized bicycle exercise test, and the influence of glucose infusion.

MATERIALS AND METHODS

Subjects

Nineteen male cadets of the Norwegian Military Academy participated in a 5-day ranger course as part of their training program. The cadets had studied at the Academy for about 1 year and were in good mental and physical condition.

The course

The activities started on a Sunday afternoon (Day 1) and finished on the following Friday afternoon (Day 6). The subjects were exposed to continuous physical activities around the clock corresponding to 35% of VO_2max or about 40,000

kJ/24 h/cadet. On the first day the subjects had a normal breakfast and lunch. During the second day the energy intake contained about 5000 kJ, the third day 3000 kJ, a cooked chicken for two cadets in the afternoon on Day 4 and only some bread (2000 KJ) on Day 5. The intake of water was free, but despite this the subjects complained of thirst during the course. The subjects had a 3–4 kg reduction of body weight during the course. No organized sleep was allowed during the whole training course, but the cadets had small periods of sleep between activities, estimated to 1–3 h during the whole course.

Bicycle exercise test

The VO_2max was estimated from two different submaximal workloads performed before the course. On Day 4 during the course and in a control experiment the cadets were tested on a bicycle ergometer with about 50% of their VO_2max during 30 min. Probably because of too short a test-time (5–10 min) the estimated VO_2max was too high. Calculated on the basis of the pulse rate response during the control experiment the relative work load was 58% of VO_2max.

Blood samples were drawn from an indwelling cannula in the antecubital vein and according to the following time schedule: 15 min before the exercise with the subjects recumbent, 0 min just before the start of the exercise with the subjects sitting on the bicycle, then after 10, 15, 20 and 30 min of exercise and during the recovery period with the subjects recumbent 35, 40, 50, 60 and 90 min after the start of exercise. The control experiment was performed 2–3 months after the course following a period with regular school activities. The pulse rate was recorded from the ear lobe and indirect brachial blood pressures were measured manometrically.

The subjects were randomly divided into two groups. Group 1 ($n = 9$) with a mean age of 24.5 years (range: 22–27) and body weight of 78.0 kg (range: 61–85) had a VO_2max of 5.0 l/min. Group 2 ($n = 10$) had a mean age of 23.5 years (range: 22–26), body weight of 79.5 kg (range: 67–90) and a VO_2max of 5.2 l/min. During the exercise test the mean workload for Group 1 was 181 Watts (range: 145–215) and for Group 2 187 Watts (range: 145–215). In contrast to Group 1, Group 2 received 25 g of glucose (20% glucose solution) by continuous infusion through a second plastic cannula in the antecubital vein from the 10th to 30th min of exercise.

Blood sampling

The blood for catecholamine analysis was collected in ice-chilled 10 ml vacuum tubes containing sodium heparin (184 usp units). The blood was kept on ice until centrifuged within 15 min in a refrigerated centrifuge. Blood for serum collection was taken in vacuum tubes, allowed to clot at room temperature for about 30 min, and then centrifuged in a refrigerated centrifuge. The plasma and serum were frozen on dry ice immediately after centrifugation and kept frozen at −80°C until analysed.

Chemical analysis

Plasma free and conjugated catecholamines were analysed with a radioenzymatic analysis according to a modification of the method of daPrada and Zürcher (1976).

To 360 μl of heparinized plasma was added 40 μl of 6N PCA (Merck, Darmstadt, F.R.G., suprapur) containing 1% EGTA and 0.1% $MgCl_2$. After 15 min centrifugation at about 2000 *g*, 100 μl of the supernatant was used in each of two parallels.

For the analysis of total catecholamines (free and conjugated) the supernatant obtained after deproteinization with PCA was diluted 1:1 with 0.3 N PCA containing 40 μl/ml of sulfatase (Sigma S-1629).

To the blank tubes 100 μl of 0.3 N PCA was added to each of three parallels. Two different standards were run. Ten μl of 0.01 N HCl containing 1 pmol of adrenaline and dopamine and 2 pmol of noradrenaline was added either to 100 μl of 0.3 N PCA (external standard) or to 100 μl of the supernatant after deproteinization of one of the samples.

To all tubes 100 μl of a freshly prepared solution was added, resulting in a final concentration of 3.2 mM dithiotreitol (Sigma, St Louis, MO), 1 M Tris–HCl, 15 mM $MgCl_2$, 1.35 mM [^{3}H]-*S*-adenosyl methionine (2 mCi) and contained 25 μl of the enzyme solution. The reaction mixture had a pH of 8.25 and was incubated at 37°C for 45 min.

The catechol-*O*-methyl transferase was prepared as described by daPrada and Zürcher (1976) but from swine liver instead of rat liver, and the enzyme was dialysed three times for 2, 8 and 4 h against a 1 mM sodium phosphate buffer pH 7.0 containing 0.1 mM dithiotreitol.

The reaction was stopped by placing the tubes on ice and by adding 150 μl ice-cold 1 nM borate buffer pH 8. In addition, 60 μl of an aqueous 50 mM tetraphenylborate (Merck) solution and 40 μl of a carrier solution containing 3 mM each of methoxythyramine, metanephrine and nor-metanephrine (all from Sigma) in 0.01 N HCl was added.

The 3-*O*-methylated amines were extracted in 4 ml of diethyl ether after shaking vigorously for 5 min. The phases were separated by centrifugation at 2000 *g*. The aqueous phase was frozen in an ethanol bath with dry ice and the ether was decanted into another set of tubes containing 100 μl of 0.1 N HCl. After 5 min with vigorous shaking the aqueous phase was frozen in an ethanol bath with dry ice and the ether phase was discarded. The aqueous phase was then washed with 4 ml of *n*-butylacetate and the aqueous phase was reduced by lyophylization for about 1 h. To make the spotting on the kiesegal plates (2.5 × 10 cm) easier, 50 μl of methanol was added to each tube. The plates were developed for 30 min in a 300-ml beaker containing 20 ml of a solvent system of chloroform:ethanol:ethylamine (16:3:2) and covered with aluminium foil. The plates were dried for about 15 min and the spots were identified by UV light. The spot corresponding to methoxytyramine was scraped directly into the scintillation vial and 10 ml of InstaGel II was added.

The spots corresponding to normatanephrine and metanephrine were scraped into polypropylene tubes and the amines were eluted from the kieselgel by shaking the tubes two times on a vortex for 5 min with 1 ml of 2 N NH_3. The amines were oxidized to vanilic acids by adding 0.25 ml of 3% $NaIO_4$ and incubated for 10 min at 37°C in a shaking water bath. After the incubation the tubes were placed in an ice-cold water bath and 0.5 ml 10 N acetic acid was added. The amines were then extracted into 6 ml of toluene. The water phase was frozen and the toluene was decanted into scintillation vials. Ten ml of InstaGel II was added and the radioactivity was counted in a scintillation counter.

Glucose was analysed with the hexokinase method (Boehringer, Mannheim, F.R.G.).

Statistics

The results are presented as Means ± SEM. When the conjugated catecholamine results did not show normal distribution, the statistical analyses were performed on logarithmically transformed data. An overall analysis of variance for repeated measures (SPSS) was used to test the overall differences within the same group and between groups. The *t*-test was used to identify significant differences. Pearson's correlation coefficient was estimated by SPSS.

RESULTS

Plasma glucose (mmol/l) (Fig. 1)

In Group 1 the plasma glucose levels did not show any significant alterations during the exercise test, but a significant increase was found from 4.6 ± 0.2 at the end of exercise to 5.1 ± 0.2 after 10 min of recovery in the control experiment ($F_{10,80} = 2.19$; $P = 0.027$). A small but significant decrease was found in the stress experiment ($F_{10,80} = 2.55$; $P = 0.010$).

In Group 2 the plasma levels of glucose rose from 4.7 ± 0.3 to 8.6 ± 0.7 during the exercise test ($F_{10,90} = 49.53$; $P < 0.0005$). During the course slightly lower plasma levels were found post exercise ($F_{10,90} = 1.79$; $P = 0.079$).

Pulse rate (beats/min) (Fig. 1)

The pulse rate increased from 54.3 ± 1.4 to 64.4 ± 1.9 when the subjects moved from the recumbent to the sitting position ($F_{1,17} = 54.27$; $P < 0.0005$). During the exercise test the pulse rate increased to 119.8 ± 2.5 after 10 min and further to 131.4 ± 2.9 after 30 min of exercise ($F_{10,170} = 464.59$; $P < 0.0005$). At the end of exercise the pulse rate decreased rapidly to 64.8 ± 2.4 after 5 min and to 57.4 ± 1.8 after 60 min of recovery before and during the course respectively. There were no significant alterations in the pulse rate response to the exercise test during the course ($F_{10,170} = 1.88$; $P = 0.051$). Intravenous glucose infusion slightly reduced the pulse rate response to exercise both in the control experiment and during the course ($F_{10,170} = 2.31$; $P = 0.015$).

Blood pressure (mm Hg) (Fig. 1)

The systolic blood pressure in the recumbent and sitting position was 120.0 ± 2.3 and 122.4 ± 3.1. The systolic blood pressure after 10 and 30 min of exercise was 171.6 ± 3.6 and 172.4 ± 5.0 ($F_{10,170} = 128.78$; $P < 0.0005$). After 5 and 60 min of recovery the systolic blood pressure had decreased to 132.6 ± 1.5 and 122.1 ± 1.8. There were no significant alterations in the systolic blood pressure response to the bicycle exercise test during the course ($F_{1,17} = 4.01$; $P = 0.061$), and there were no significant differences between the two groups ($F_{1,17} = 3.44$; $P = 0.081$). [The *t*-test, however, showed significantly higher systolic blood pressure during the course

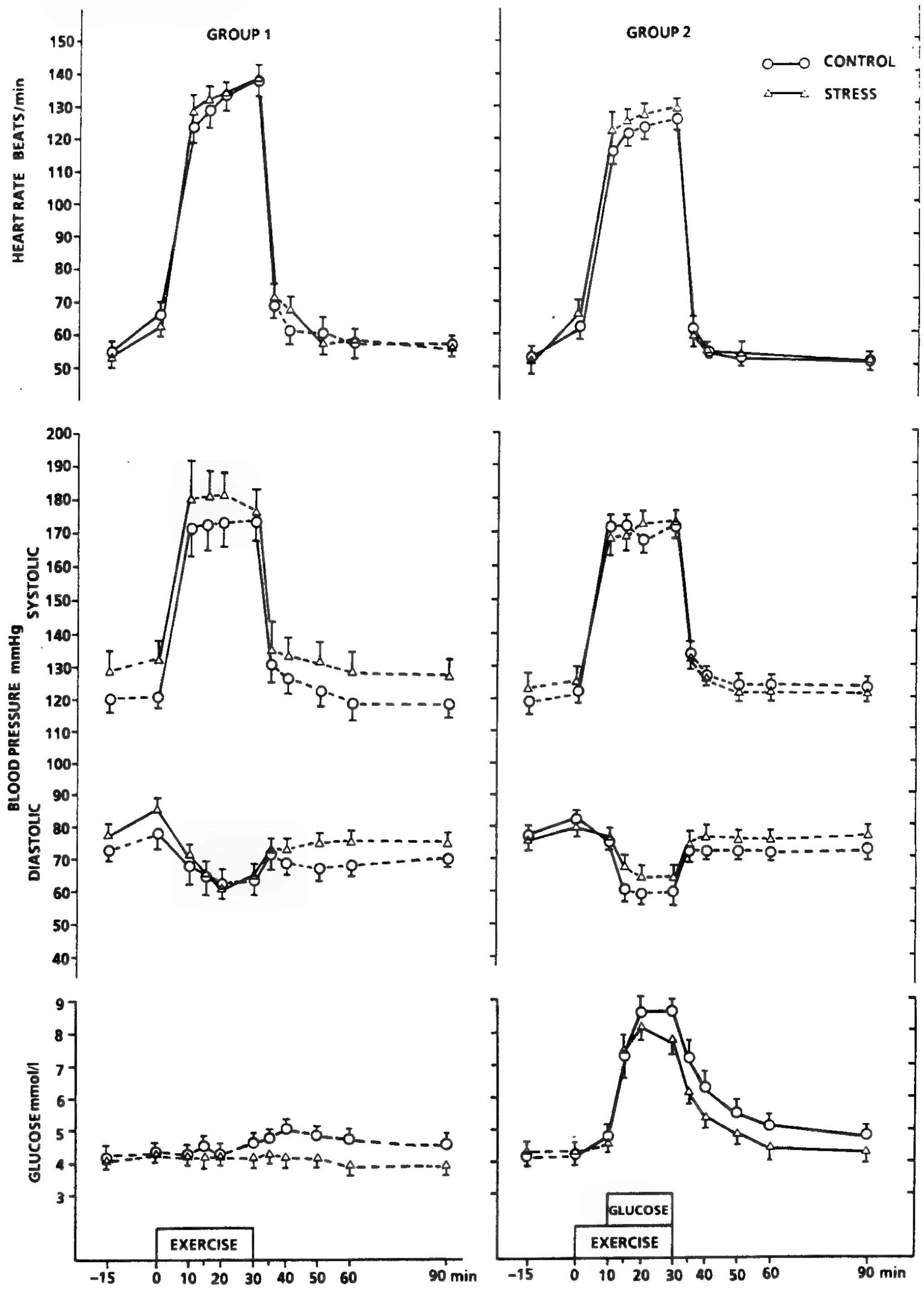

Figure 1. The pulse rate, blood pressure and plasma glucose responses to 30 min ergometer exercise with approximately 60% of VO_2max in a control experiment and after a 5-day ranger training course with heavy physical exercise combined with energy and sleep deprivation. In contrast to Group 1 the subjects of Group 2 received 25 g of glucose intravenously during the last 20 min of exercise. The levels are presented as means and the vertical bars represent the standard error of the mean. Point to point variations significant at $P<0.01$ are indicated by thick lines.

compared to the control experiment in Group 1 at time points 50 min ($P = 0.002$), 60 min ($P = 0.015$) and 90 min ($P = 0.046$).]

The diastolic blood pressure in the recumbent and sitting position was 75.3 ± 1.6 and 80.5 ± 1.9 ($F_{1,17} = 5.92$; $P = 0.026$). The diastolic blood pressure decreased during the exercise test to 68.4 ± 3.0 after 10 min of exercise and further to a minimum of 60.8 ± 2.4 after 20 min of exercise ($F_{10,170} = 19.26$; $P < 0.0005$). After 5 and 60 min of rest the diastolic blood pressure was 71.5 ± 1.1 and 71.1 ± 1.0. There was a small but significant increase in the diastolic blood pressure during the course ($F_{1,17} = 4.46$; $P = 0.05$), whereas no significant differences were found between the two groups.

Plasma free noradrenaline (nmol/l) (Fig. 2)

A significant increase was found in the plasma noradrenaline levels during the course ($F_{1,16} = 16,94$; $P = 0.0015$). By changing the body position from recumbent to the sitting position noradrenaline increased from 1.3 ± 0.2 to 1.5 ± 0.2 in the control experiment and from 3.4 ± 0.4 to 4.0 ± 0.4 during the course ($F_{1,16} = 5.71$; $P = 0.030$). There were no significant differences in this response between the two groups or from the control experiment to the course.

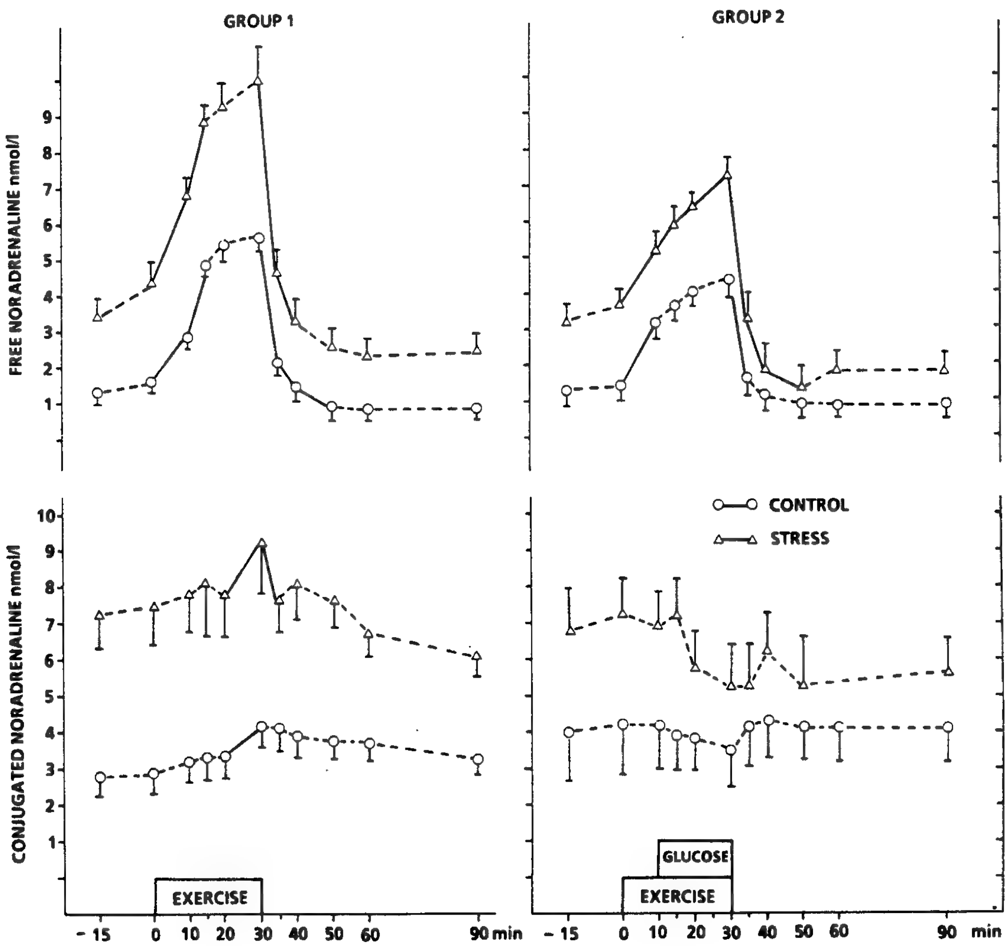

Figure 2. Plasma levels of free and conjugated noradrenaline. For details, see Fig. 1.

A gradual increase was seen during the exercise test in the control experiment to a maximum level of 5.6 ± 0.8 after 30 min in Group 1 ($F_{10,160} = 58.79$; $P = 0.0005$) and a significantly lower response to 4.2 ± 1.2 in Group 2 ($F_{10,160} = 2.15$; $P = 0.024$). During the course a significantly increased response was observed after 30 min of exercise to 10.0 ± 1.5 in Group 1 and 7.3 ± 1.8 in Group 2 ($F_{10,160} = 4.44$; $P < 0.0005$). After 5 and 60 min of rest the plasma noradrenaline levels in Group 1 were 2.1 ± 0.3 and 0.8 ± 0.1 in the control experiment and 4.6 ± 0.6 and 2.4 ± 0.4 during the course. In Group 2 the levels after 5 and 60 min of rest were 1.6 ± 0.4 and 0.9 ± 0.2 in the control experiment and 3.3 ± 0.7 and 1.7 ± 0.3 during the course.

Plasma conjugated noradrenaline (nmol/l) (Fig. 2)

The plasma levels of conjugated noradrenaline increased during the course from 3.4 ± 0.7 in the control experiment to 6.6 ± 0.7 during the course ($F_{1,16} = 44.17$; $P < 0.0005$). The small but significant increae found during the bicycle exercise test in Group 1 ($F_{10,80} = 2.29$; $P = 0.020$), but not in Group 2, was not changed during the course ($F_{10,80} = 1.69$; $P = 0.096$).

Plasma free adrenaline (nmol/l) (Fig. 3)

The plasma levels of free adrenaline increased from 0.17 ± 0.03 in the control experiment to 0.41 ± 0.07 during the course ($F_{1,16} = 27.18$; $P < 0.0005$). The maximal levels were reached after 30 min of exercise; 0.63 ± 0.09 in the control experiment and 1.43 ± 0.19 during the course ($F_{10,160} = 67.72$; $P = 0.0005$). A

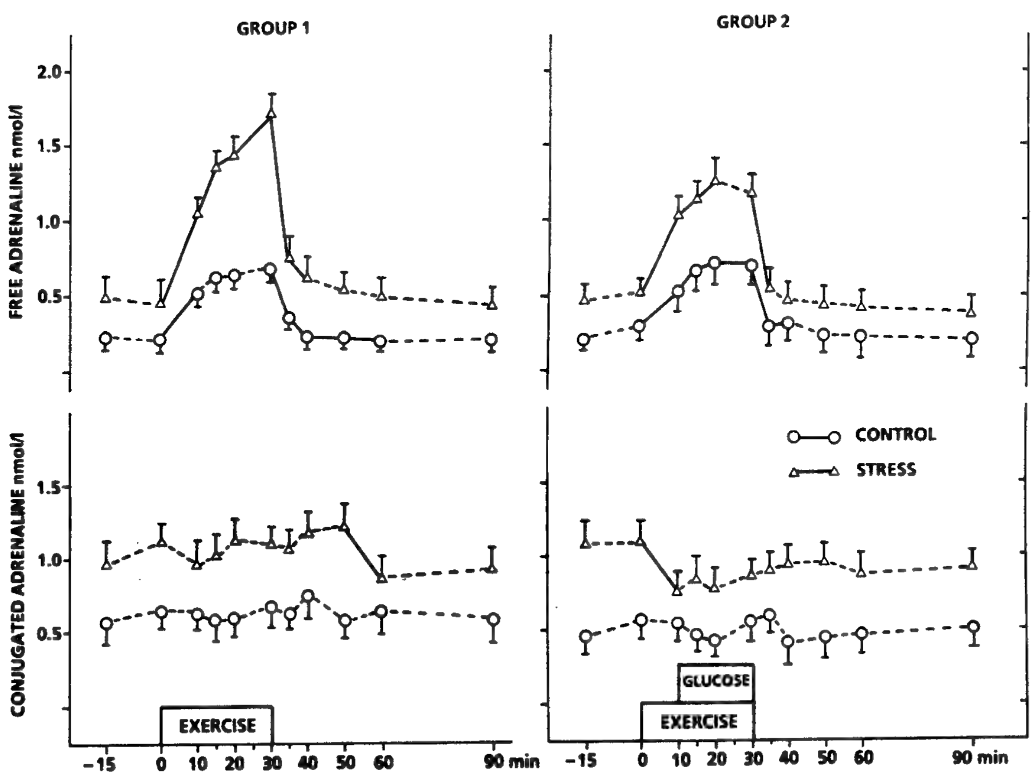

Figure 3. The plasma free and conjugated adrenaline. For details, see Fig. 1.

significant increase in the response to bicycle exercise was found during the course ($F_{10,160}=7.47$; $P<0.0005$). No significant difference was found between the two groups.

Plasma conjugated adrenaline (nmol/l) (Fig. 3)

Plasma conjugated adrenaline concentration increased during the course from 0.46 ± 0.08 to 1.08 ± 0.15 ($F_{1,16}=14.97$; $P=0.001$). There were no significant alterations during the exercise test, either in the control experiment or during the course. There were no significant differences between the two groups.

Plasma free dopamine (nmol/l) (Figs 4 and 5)

One person showed extremely high plasma levels of both free and conjugated dopamine in the control experiment. The results from this subject are therefore presented separately (Fig. 5), making the results from the rest of the subjects (Fig. 4) normally distributed.

The plasma levels of free dopamine increased from 0.20 ± 0.03 to 0.42 ± 0.06 during the course ($F_{1,16}=40.94$; $P<0.0005$). The plasma free dopamine response to the bicycle exercise test ($F_{10,150}=17.45$; $P<0.0005$) was significantly increased during the course ($F_{10,150}=4.48$; $P<0.0005$). Significantly lower plasma free dopamine levels were found during the exercise test in the subjects receiving glucose intravenously both in the control experiment ($F_{1,15}=2.87$; $P=0.003$) and during the course ($F_{10,150}=3.13$; $P=0.001$). These results were confirmed with non parametric statistics with all the subjects included.

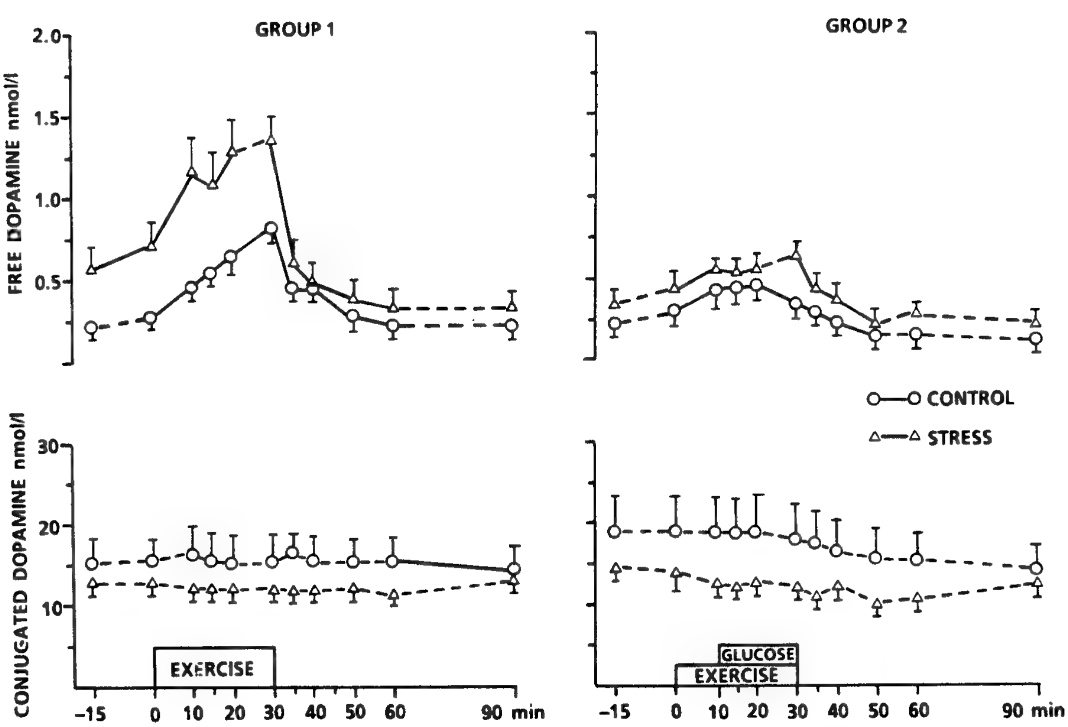

Figure 4. Plasma free and conjugated dopamine. For details, see Fig. 1.

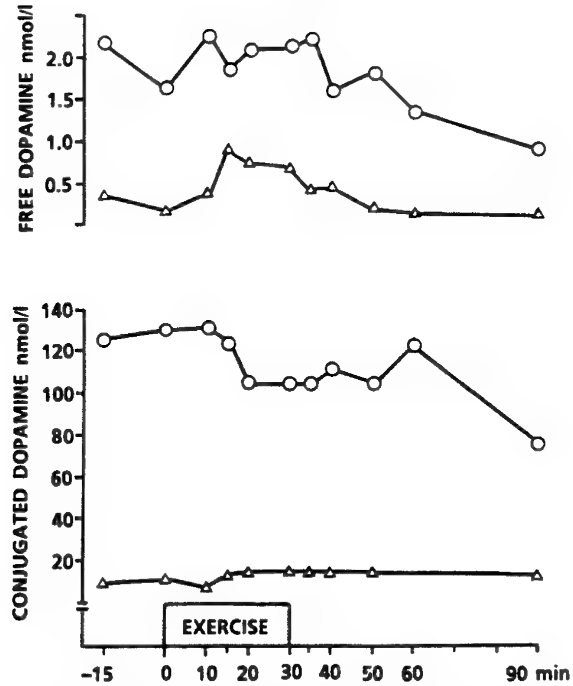

Figure 5. The results from one cadet with extremely high precourse levels of both free and conjugated dopamine. For details, see Fig. 1.

Plasma conjugated dopamine (nmol/l) (Figs 4 and 5)

The plasma concentration of conjugated dopamine was 17.3 ± 4.2 in the control experiment and 14.4 ± 1.6 during the course. The slight decrease found during the exercise test ($F_{10,160} = 2.87$; $P = 0.003$) was reduced during the course ($F_{10,160} = 2.89$; $P = 0.002$). There were no significant differences between the two groups, either in the control experiment or during the course. The great interindividual variations found in the control experiment were strongly reduced during the course as shown in Fig. 6.

Correlations

The significant correlation found between free and conjugated dopamine in the control experiment decreased from $R = 0.84$ ($P < 0.001$) to 0.62 ($P < 0.01$) during the 30 min of exercise and returned to $R = 0.86$ ($P < 0.001$) after 60 min of recovery. No significant correlations were found during the course. Significant correlations were also found between the plasma concentrations of noradrenaline and adrenaline before and during the exercise tests both in the control experiment ($R = 0.66$, $P < 0.001$) and during the course ($R = 0.66$, $P < 0.001$). Between noradrenaline and dopamine, significant correlations were found only in the control experiment ($R = 0.61$; $P < 0.01$).

DISCUSSION

This paper confirms our previous findings of an increased plasma catecholamine response to a standardized exercise test without alterations in the pulse rate

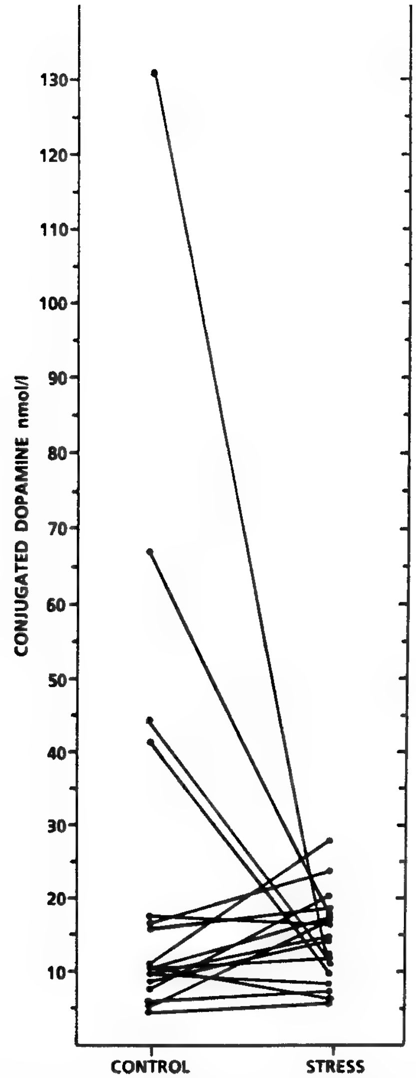

Figure 6. Individual pre-exercise conjugated dopamine levels from the control experiment and after 5 days of physical strain, sleep and energy deficiency.

response after prolonged strain, sleep and energy deficiency (Opstad *et al.*, 1980; Opstad, 1990). It also shows that this increase in the plasma catecholamine response is followed by only moderate alterations in systolic and diastolic blood pressure responses. The results therefore imply that there is a peripheral adrenergic desensitization, which could explain the performance reduction or exhaustion during the ranger training course. It has been shown in animal studies, however, that during more prolonged stress there is also an exhaustion of the nervous system or the endocrine glands (Selye, 1946). The present findings are in accordance with the fact that the number and the affinity of the β_2-receptors on mononuclear leucocytes and granulocytes are strongly reduced during a similar course (Opstad *et al.*, 1990).

Studies of the effects of fasting or calorie restriction on catecholamines have been remarkably inconsistent. Reduced tissue noradrenaline turnover was found in the heart, liver, spleen and pancreas of the rat (Young and Landsberg, 1977; Landsberg and Young, 1978; Avakian and Horwath, 1981; Young *et al.*, 1984). Garty *et al.*

(1989) observed an increase in plasma adrenaline, but no alterations in plasma noradrenaline, after 24 h fasting in the rat. Weick *et al.* (1983) did not find any alterations in plasma arterial catecholamines, whereas Avakian and Horwath (1981) reported a decrease after 24 h fasting. In humans, Bennet *et al.* (1984) found a non significant decrease in plasma noradrenaline after 48 h fasting, whereas Galbo *et al.* (1981) and Pequignot *et al.* (1980) found increases both in plasma adrenaline and noradrenaline levels after 59 and 15 h of fasting respectively. The present results show that glucose slightly reduces the noradrenaline and pulse rate responses to ergometer exercise, in agreement with other previous studies (Andersson *et al.*, 1988; Opstad, 1990).

This difference in the noradrenaline and pulse rate response to bicycle exercise between the two groups can probably not be explained by different workloads in the two groups, because the subjects were divided into groups at random, and the absolute workload was highest in the group with the lowest responses (186 Watts for Group 1 and 181 Watts for Group 2). Comparisons between the control and stress experiment are not affected by this, because the absolute workloads on a data controlled ergometer bicycle in the two experiments were the same.

A small increase found for conjugated noradrenaline during ergometer exercise in Group 1 was reversed by glucose infusion in Group 2. No significant alterations were found for conjugated adrenaline during short-term exercise neither in the control experiment nor during the course, whereas a small decrease was found in conjugated dopamine which was reduced during the course. Sothmann *et al.* (1987) similarly found increased plasma levels of conjugated noradrenaline after exercise exceeding 60% of maximal VO_2 for 8 min. In contrast, Häggendal (1963) did not find any alterations in the conjugated catecholamines during acute physiological stimulation, and Joyce *et al.* (1982), and Vandongen (1984) found a small decrease in the conjugated catecholamines when the subjects were exposed to bicycle exercise for 12 min.

In contrast to the very small variations in the conjugated catecholamines during short-term ergometer exercise, a 2–3-fold increase was found in plasma noradrenaline and adrenaline during the ranger training course. This means that during short-term exercise there are only minor variations in plasma conjugated catecholamines, whereas during prolonged strenuous exercise plasma conjugated adrenaline and noradrenaline increase proportional to the free adrenaline and noradrenaline. This indicates a certain role of sulfoconjugation for the inactivation of sympathoadrenally released catecholamines during prolonged stress, since no conjugated amines were furnished from other sources.

The significant correlation between the plasma levels of free and conjugated dopamine in the control experiment indicates that sulfated dopamine may be a source for free plasma dopamine. The small increase found for most subjects in conjugated dopamine during the course (Fig. 6) also indicates a certain role for sulfoconjugation in the inactivation of free dopamine. The significant correlations between the free catecholamines suggest a certain common mechanism of release.

The physiological significance of plasma conjugated catecholamines is still not established. It has been shown that sulfated dopamine can serve as a direct precursor in the synthesis of noradrenaline (Buu and Kuchel, 1979). Since there was no significant decrease in the plasma concentration of conjugated catecholamines during the ergometer exercise test, there is no reason to believe that the conjugated

catecholamines are the source of the increased levels of free catecholamines during short-term exercise. During the course both free and sulfated adrenaline and noradrenaline increased to the same extent. It thus appears that conjugated catecholamines do not serve as a pool for the free amines during prolonged stress.

The phenol-sulfo-transferase is present in many tissues with particularly high concentrations in the liver, the gastrointestinal tract and the blood platelets. Ingested catecholamines in nutrients are converted to conjugated catecholamines in the gastrointestinal tract before they are released into the circulation. Some nutrients such as fruits and juices contain high concentrations of catecholamines (e.g. one banana contains 1 mg of dopamine) (Waalkes *et al.*, 1958; Crout and Sjoerdsma *et al.*, 1959; Udenfriend *et al.*, 1959; Davidson *et al.*, 1981; Tyce *et al.*, 1986). The increase in plasma conjugated catecholamines in the blood and the urine is less when the catecholamines are given intravenously than when they are absorbed through the gastrointestinal tract (Kopin, 1985; Peyrin, 1986; Cuche *et al.*, 1986), showing that the nutrients and sulfotransferase in the gastrointestinal tract are important for the plasma levels of conjugated catecholamines. It is still unclear what role sulfoconjugation plays in the inactivation of catecholamines from the sympatho-adrenal system.

Great interindividual variations in the plasma levels of conjugated dopamine have been observed (Peyrin *et al.*, 1986). It has been discussed whether this variability is due to genetic factors or environmental factors such as nutrients. In addition to the lack of alterations of the plasma levels of conjugated dopamine during the course, there was also a decrease in the standard deviation of the results as shown in Fig. 6. The reason for the great interindividual variability in the control experiment therefore has to be environmental rather than genetic factors. During the course the subjects were allowed only about 3000–4000 kJ/day, mostly in the form of slices of bread. The normal food intake is therefore probably the origin of the great interindividual variations seen in the control experiment. It is assumed that 12 h of fasting is enough to eliminate the influence of nutrients on the plasma and urinary levels of conjugated catecholamines (Kuchel *et al.*, 1982) since the half life is about 40–60 min. In the present study the control experiment was performed after one overnight fast, which was still not enough to eliminate the variability of the plasma conjugated catecholamines.

In conclusion, prolonged physical strain leads to a peripheral adrenergic desensitization which may be a limiting factor for performance. There is an increase in the conjugated adrenaline and noradrenaline during prolonged physical strain but not during short-term exercise. The conjugated catecholamines are not deconjugated to free catecholamines during exercise.

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PAPER III

Biogenic Amines, Vol. 10, No. 4, pp. 329–344 (1994)

The dynamic response of the β2- and α2 adrenoceptors in human blood cells to prolonged exhausting strain, sleep and energy deficiency

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Leucocyte β_2-receptors and platelet α_2-receptors were studied during a five days ranger training course with heavy physical activities day and night, energy deficiency and almost without sleep. The β_2-adrenoceptors on granulocytes and mononuclear cells decreased to a minimum density and affinity after 2 and 4 days of activities respectively. For the rest of the course the β_2-receptors increased, however, without reaching control values at the end of the course. A significant decrease of about 15 % was found on day 3 in both platelet α_2-receptor density and affinity followed by an up-regulation to about 20% above control levels on day 5. A significant correlation ($r= -0.6$ to -0.8) between the β_2-receptor density and affinity, and the plasma catecholamines was recorded during the first 2-3 days, indicating a homologous down-regulation. The regeneration of receptors in spite of high catecholamines reflects a predominance of heterologous up-regulation during the second half of the course. The results indicate that β-adrenergic receptor down-regulation contributes to attenuation of the adrenergic responsiveness during the first 2-3 days of exhausting physical activities, but is not sufficient to account for the desensitization seen after longer periods of stress.

Key words: stress, exercise, sleep deprivation, energy deficiency, adrenoceptor and catecholamines.

INTRODUCTION

The physiological and molecular mechanisms leading to exhaustion or reduced physical and mental performance after prolonged exercise, sleep and energy deficiency are scarcely investigated. Previously we have found that the plasma catecholamine response to a standardized bicycle exercise test was strongly increased during prolonged stress without any similar increase in pulse

rate and blood pressure response (Opstad *et al.*, 1980, Opstad 1990, 1991). This indicates an attenuation of vascular responsiveness to catecholamines during stress and that the desensitization is partly compensated by increased catecholamine output. The mechanisms for this desensitization may be receptor down-regulation or uncoupling. Human tissues are not easily available for adrenergic receptor studies. However, for the β_2- and α_2- adrenergic receptors, much information has been gained from studies of peripheral blood leucocytes and platelets. Down-regulation of ß-adrenergic receptors has been demonstrated on isolated human cells in vitro (Johnson *et al.*, 1978, Tashkin *et al.*, 1982), in vivo during medical treatment with pharmacological doses of adrenergic agents (Aarons *et al.*, 1980, Galant *et al.*, 1978, Tomah and Cryer, 1980), and under pathological conditions (Collucci *et al.*, 1981, Sano *et al.*, 1981, Tsujimoto *et al.*, 1984, De Biasi *et al.*, 1986). Up-regulation of the receptor takes place when the receptor is blocked by an antagonist (Aarons *et al.*, 1980). Conflicting results exist whether the α_2-receptors on platelets are regulated by the plasma catecholamine concentrations. The down-regulation shown for the α_2-receptor in vitro (Cooper *et al.*, 1978) was possibly due to agonists retained in the platelets, and was not reproduced by [^{3}H]yohimbine-binding studies (Karliner *et al.*, 1982). However, down-regulation of the α_2-adrenergic receptor has been demonstrated in patients suffering from pheochromocytoma (Chobanian *et al.*, 1982, Snavely *et al.*, 1982, 1983, Brodde *et al.*, 1984) and an up-regulation was found in patients suffering from anorexia nervosa and bulemia (Heufelder *et al.*, 1985). The adrenoceptors can be heterologously regulated by thyroid hormones, glucocorticoids and gonadal steroids (Fraser *et al.*, 1980, Ginsberg *et al.*, 1981, Motulsky *et al.*, 1982, Nahorski *et al.*, 1982, Stiles *et al.*, 1984). Dietary sodium influences the adrenergic receptor density and response (Mulvihill-Wilson and Pettinger, 1981, Volpe *et al.*, 1982). The effect of acute exercise or endurance training on the adrenergic receptors is a matter of dispute (FitzGerald *et al.*, 1981, Hollister *et al.*, 1981, Burman *et al.*, 1985).

The main purpose of the present paper was to study the mechanisms of the adrenergic desensitization during prolonged exhausting exercise lasting for several days.

MATERIALS AND METHODS

Subjects

The subjects of this investigation were the cadets of the Norwegian Military Academy participating in a ranger training course as a part of their ordinary education program. The cadets were between 20 and 25 years of age, well trained and motivated. The courses lasted from Sunday afternoon (day 1) until Friday morning (day 6). The subjects were exposed to continuous physical activities around the clock, corresponding to about 35 % of $VO_{2\ max}$ or a calorie consumption of

about 40.000 kiloJoule(kJ)/24 hours/cadet. The energy intake varied from day to day with a normal breakfast and lunch on Sunday before the start of the course, 5000 kJ on the second day, 3000 kJ on day 3, a cooked chicken in the afternoon on day 4 and only some bread (2000 kJ) on day 5. The intake of water was free, but in spite of this the subjects complained of thirstiness. The subjects had a 3-4 kg reduction in body weight during the course.

There was no organized sleep during the course. However, observations by the investigators and officers and heart rate recordings indicated that each cadet slept 1 to 3 hours during the whole course.

Blood cells for β_2-receptor analysis were obtained from twelve cadets, six in each of two identical courses. Platelets for the α_2-receptor binding studies were obtained the following year from 5 and 6 cadets in two identical courses.

Blood Sampling

The blood sampling took place between 06^{00} and 08^{00} in the morning after 30 min of rest in the sitting position. The first blood sample was collected on Monday morning after about 15 hours of activities. For the β_2-receptor study 40 ml of blood was drawn from each cadet every day into vacuum tubes containing heparin and trasylol. For the α_2-receptor study 60 ml was drawn each day in vacuum tubes containing EDTA and trasylol. The tubes were put on ice immediately, then centrifuged, and the plasma was removed and frozen on dry ice. The remaining cell suspension was kept on ice and transported (2-4 hours) to the laboratory for further analysis.

Cell Preparation

The cell suspensions were diluted to their original sample volume with 0.15 M NaCl. The isolation of the mononuclear cells (lymphocytes and monocytes) and the granulocytes was performed according to the method described by Bøyum (1968). The resulting cell fraction was centrifuged at 600 x g for 5 minutes, and resuspended in 6 ml of a buffer containing 50 mM Tris, 10 mM $MgCl_2$ and 30 mM NaCl at pH=7.4. The cell concentration was determined by an electronic cell counter.

Preparation of Platelets

EDTA/trasylol blood was centrifuged at 300 x g for 12 minutes (20°C). The platelet-rich plasma was further centrifuged at 200 x g for 5 minutes (20°C) to remove remaining cells. The platelets were then pelleted (10 min, 800 x g, 4°C) and washed twice at 4°C in 50 mM Tris (pH 7.5) with 100 mM NaCl and 5 mM EDTA, finally resuspended in the same buffer and counted. The

concentrations were in the range from 0.4 to 0.8 million platelets/µl. The platelet suspension contained 0-1 leucocyte/µl (N=30).

β_2 Adrenoceptor Binding Assay

^{3}H-Hydroxybenzylisoproterenol (HBI) (specific activity 26.9 Ci/mmol) was obtained from New England Nuclear Corporation. Binding experiments were performed by incubating 5×10^6 mononuclear cells or granulocytes with various concentrations (1-10 nM) of HBI in a total volume of 0.5 ml incubation buffer for 15 minutes at 37° C. The incubation buffer had the following composition: 50mM Tris-HCl; 10mM MgCl2; 30 mM NaCl; 0.8 mM ascorbic acid (to protect HBI and the catecholamines from oxidation) and 0.1 mM GTP; pH=7.4. Each cell suspension was tested in triplicate, and the white blood cells were intact in this buffer.

In the competition experiments varying concentrations of adrenaline (10^{-8} to 10^{-3}) were added to the cell suspension, containing 10 nM HBI. In each experiment "nonspecific binding" was determined by measuring the amount of radioactivity retained in the samples in the presence of a high concentration of adrenaline (10^{-3} M). Specific binding was defined as the total binding minus the tracer not displaced by 10^{-3} M adrenaline. Incubations were terminated by centrifuging the incubation tubes at 1200 x g for 10 minutes at 2°C. The cell pellet was then washed tree times with ice-cold incubation buffer, and finally dissolved in 0.3 ml 10% Na-dodecyl-sulfate, decanted into scintillation vials and counted.

The dissociation constant (Kd) for HBI and the total number of binding sites (Bmax) were determined by analysis of Scatchard plots resulting from the saturation curves for HBI. Receptor affinity for adrenaline to granulocytes was derived from the adrenaline-competition experiment where the HBI-concentration was 10 nM. This affinity was expressed as the logarithm of the adrenaline concentration giving half maximal displacement of HBI (ID50).

α_2 Receptor Binding Assay

In the α_2-receptor binding studies, 40 to 80 million platelets were incubated with at least 8 different concentrations of [^{3}H]-yohimbine (82,7 mCi/mmol) from 0.5 to 20 nM with and without 1 mM phentolamine. Specific binding was defined as the tracer binding which could be displaced by 1 mM of phentolamine. Incubations were done in duplicate at 37°C for 20 min. Bound and free radioactivity was separated by a filter method using a Skatron cell harvester. Binding data were calculated by Scatchard analysis, which in every experiment showed a linear pattern. Calculation of Kd and Bmax were based on linear regression of at least 8 data points.

Receptor affinity for noradrenaline to platelets was derived from a noradrenaline competition experiment where the [^{3}H]-yohimbine concentration was 10 nM. This affinity was expressed as the logarithm to the noradrenaline concentration giving half maximal displacement of [^{3}H]-yohimbine (ID50).

Catecholamine Analysis

The blood samples for analysis of catecholamines were collected in ice-chilled vacuum tubes containing heparin. The tubes were stored on ice until centrifuged within 30 minutes in a refrigerated centrifuge. After 20 minutes of centrifugation at 3000 x g the plasma was frozen on dry ice and kept at -80°C until analyzed. The catecholamines (noradrenaline, adrenaline and dopamine) were determined by a radioenzymatic method (Da Prada and Zürcher 1976).

Statistics

The results are presented as means ± SEM. An analysis of variance for repeated measures were used to test overall differences during the course (BMDP,4V). Differences from day to day were estimated by the Student T-test (BMDP,3D). The linear correlation coefficient was calculated by the method of least squares.

RESULTS

The binding of HBI to human mononuclear cells and granulocytes showed the characteristics of specific binding. Binding was saturable (Fig. 1) and reversible (Fig. 2).

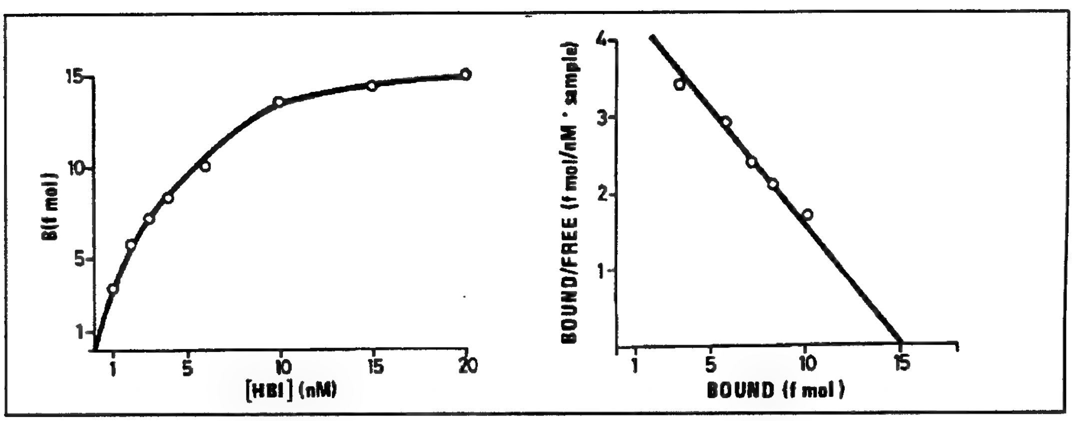

Figure 1. Left curve: Specific binding (B) of [^{3}H]-Hydroxybenzylisoproterenol (HBI) to mononuclear cells as a function of the HBI-concentration in the incubation medium containing 5x10^6 cells. Each point represents the mean of three experiments. Right curve: Scatchard plot obtained from the results in the upper curve. This yields a strait line, indicating a single class of binding sites.

Moreover, competition with agonists corresponded to the known order of potency for β_2-adrenergic receptors: Isoprenaline > adrenaline > nor-adrenaline. The binding was also strictly stereospecific. Scatchard analysis of the binding-data yielded a straight line indicating a single class of binding sites (Fig. 2). This curve gives the dissociation constant (Kd) for HBI as the negative reciprocal of the slope of the line, whereas the total number of receptors bound (Bmax) is found from the first axis intercept. Similar data were obtained for the α_2-receptor binding to platelets.

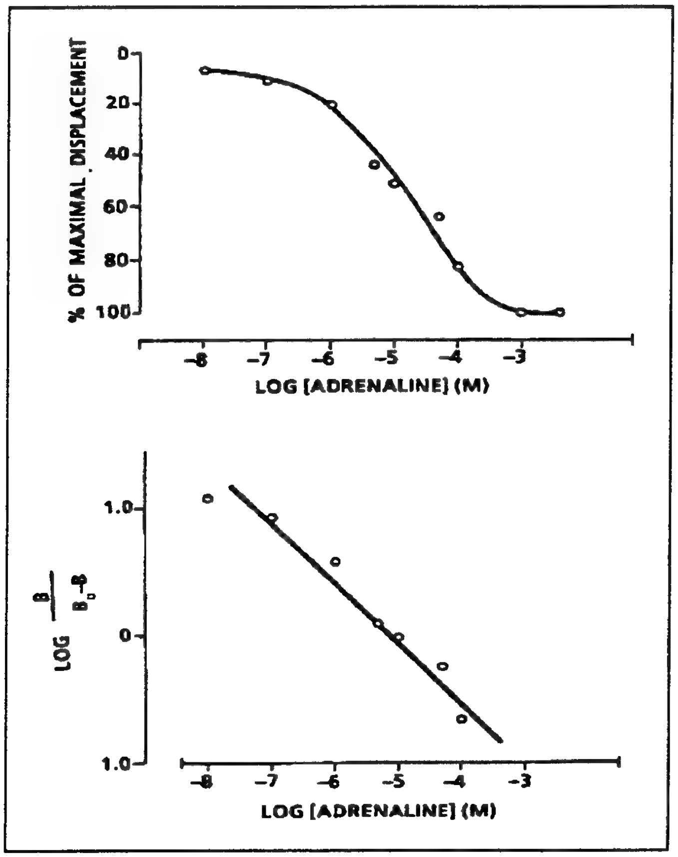

Figure 2. Competition of unlabelled adrenaline for HBI-binding sites on mononuclear cells. The HBI-concentration was constantly 10 nM. Maximal displacement of HBI-concentration was reached at 10^{-3} M. The adrenaline concentration giving half maximal displacement of HBI (ID50) was found from these experiments.

Mononuclear Cells

The density of β_2-adrenergic receptors on mononuclear cells decreased to a minimum level of 45 % of the control levels on day 4 of the course ($F_{4.58,50.43}$=65.87; p<0.00005) (Fig. 3). On the last day (day 6) the number of binding sites returned to the same level as on day 2. These values were, however, significantly lower (T_{10}=6.10; p=0.0001) than the control values. The apparent Kd for specific HBI-binding showed a small increase from day 2 during the stress period ($F_{5.28,58.08}$=17.58; p<0.00005). In the control experiment the mononuclear cells consisted of about 20 % monocytes and 80 % lymphocytes. From day 2 and during the rest of the course the

number of monocytes were increased to 40-50% with a concomitant decrease of lymphocytes to 50-60%.

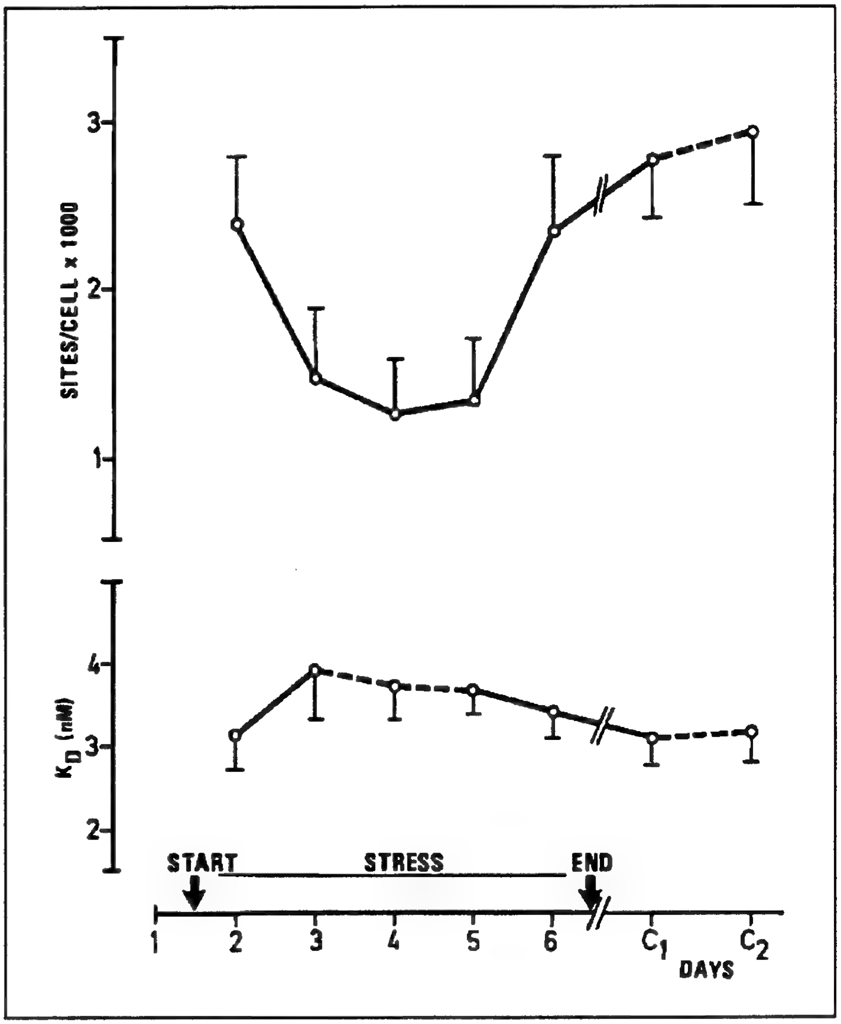

Figure 3. The upper curve shows the total number of HBI-sites on mononuclear cells each day during the ranger training course with heavy physical exercise, energy and sleep deficiency. C_1 and C_2 are the control values obtained several weeks after the course. Values are given as means and SEM. Day to day variations statistically significant at P< 0.01 are indicated by thick lines and not significant changes by dotted lines. The dissociation constant Kd is shown in the lower curve.

Granulocytes

The total number of β_2-receptors on granulocytes decreased to a minimum of 60 % of control levels on day 3 ($F_{4.83,53.10}$=27.41; p<0.00005) (Fig. 4). From then the number increased gradually, but was still below control value on day 6 (F_{10}=4.57; p<0.0010). Bmax was reduced by about 45 % during the course. On the other hand the dissociation constant (Kd) was only slightly increased on the second and third day of the stress period ($F_{3.51,38.57}$=8.48; p<0.0001). The ID50 value for adrenaline displacement of HBI had a maximum value on day 3 and day 4 and was

partly reversed on the following days without reaching control levels (equal to day 2) ($F_{6,66}=22.69$; $p<0.00005$) (Fig. 5).

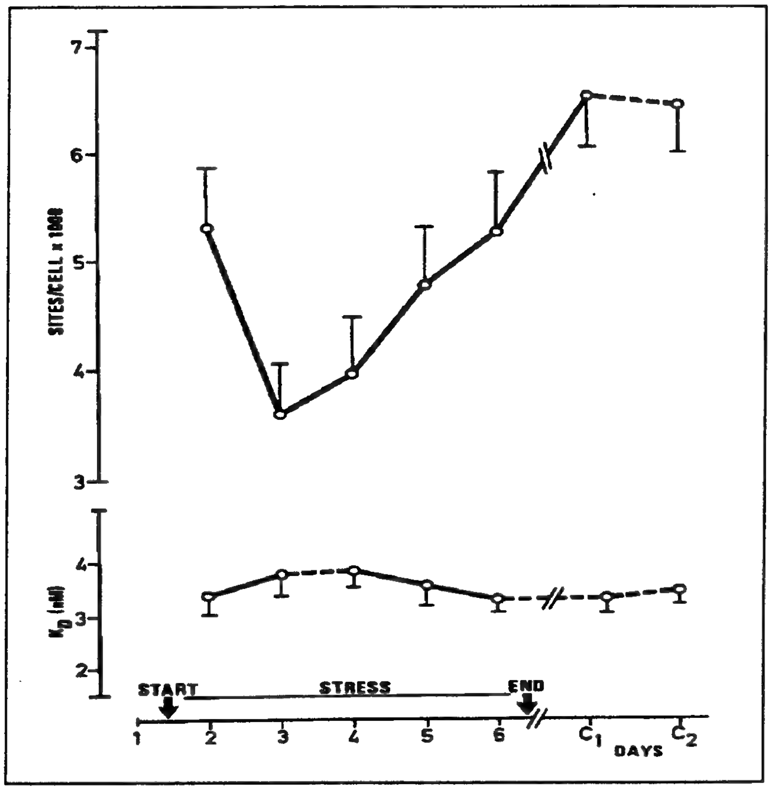

Figure 4. Total number of HBI-sites, and values for the dissociation constant Kd for granulocytes during the ranger training course. For details see Fig. 3.

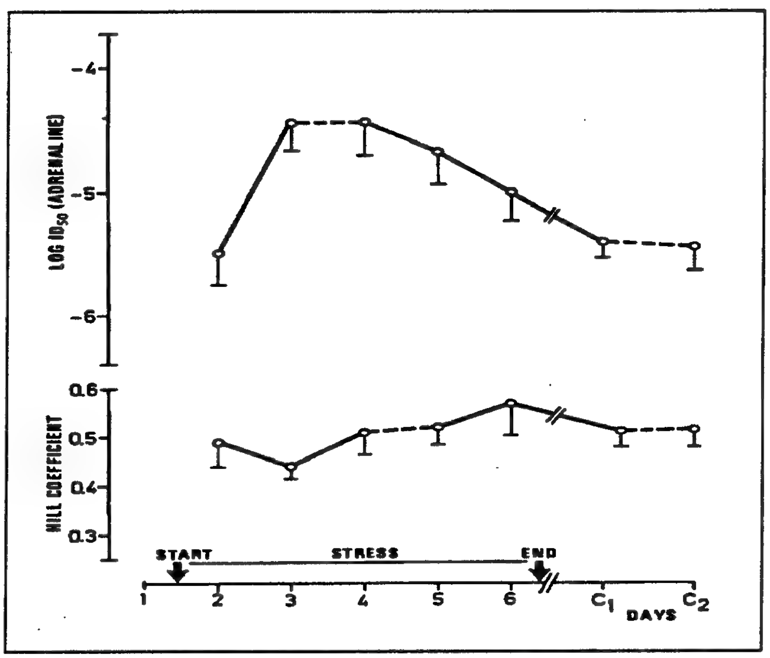

Figure 5. Results from the adrenaline-competition experiment on granulocytes. The ID_{50} values are the adrenaline concentrations giving half maximal displacement of 10 nM HBI. $5x10^6$ granulocytes were present in the incubation medium. In the lower curve the means of the Hill coefficients are given for each day during the course. For details see Fig. 3.

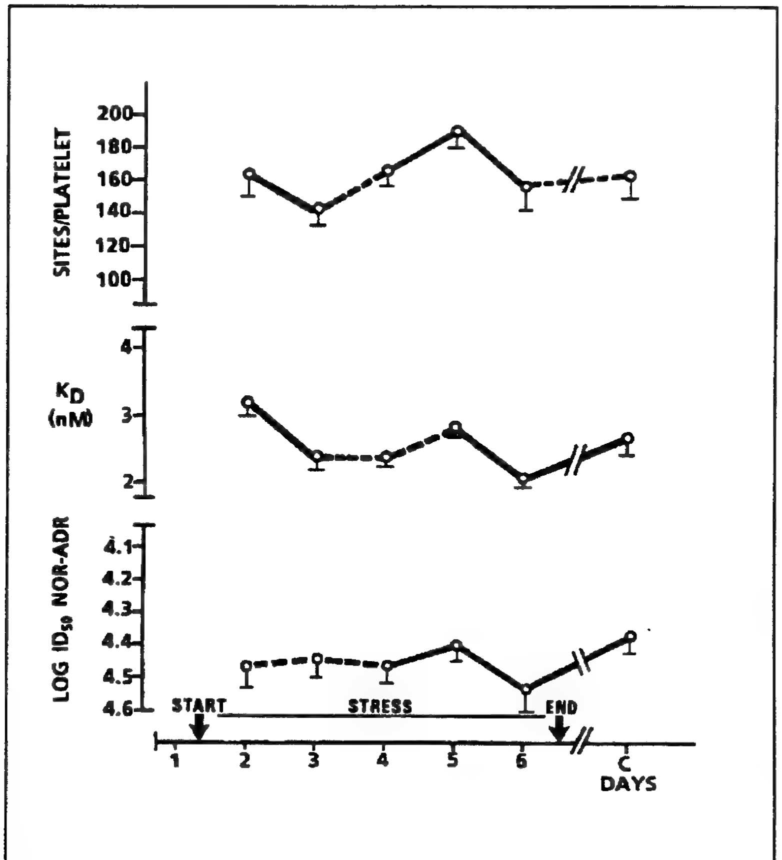

Figure 6. Total number of [^{3}H]-yohimbine binding sites on platelets during the ranger training course (upper trace). In the middle curve the dissociation constant (Kd) is shown. The results from the noradrenaline displacement curve on platelets are given in the lower curve.

Platelets

A significant reduction of about 15 % was found in the platelet α_2-receptor number on day 3 (T_{10}=1.29; p<0.0336) followed by an increase to about 20 % above control level on day 5 (T_{10}=4.59; p<0.0010) (Fig. 6). The apparent Kd was increased on day 1 and was reduced during the rest of the course ($F_{3.47,34.68}$=5.33; p=0.0028). ID50 for noradrenaline was reduced during the course ($F_{5,6}$=4.97; p=0.0381).

Catecholamines

All plasma catecholamines were increased during the course. Noradrenaline increased 3-4 fold ($F_{4.51,45.05}$=17.37; p<0.00005), adrenaline ($F_{4.32,43.25}$=9.64; p<0.00005) and dopamine ($F_{5.56,55.58}$=7.78; p<0.00005) were doubled (Fig. 7). These results were obtained from the subjects analyzed for ß$_2$-receptors and are similar to those achieved during previous courses (Opstad *et al.*, 1980, Opstad and Aakvaag, 1983)

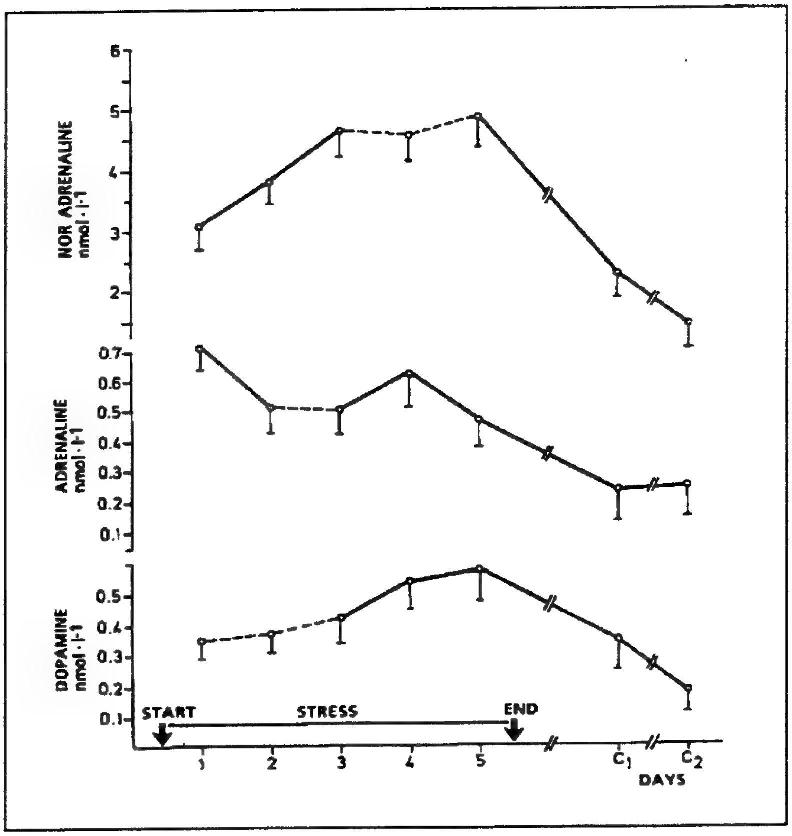

Figure 7. Changes in the plasma concentrations of noradrenaline, adrenaline and dopamine during the ranger training course. For details see Fig 3.

Correlations

Correlation coefficients recorded during the first three days of the course are given in Table 1: For the β_2-adrenoceptors there was a highly significant correlation between the receptor density and affinity indicating common regulatory mechanisms. There was also a significant but weaker negative correlation between receptor density and plasma catecholamines. For the granulocytes there was also a correlation between receptor number and log ID50.

β_2-Receptor density of	Adrenaline	Nor-adrenaline	Dopamine	Kd	Log ID50
Lymphocytes	-0.57	-0.78	-0.59	-0.92	--
Granulocytes	-0.64	-0.73	-.047	-.087	-0.87

Table 1 Shows the correlation coefficients (r) between β_2-adrenoceptor density and plasma catecholamines, Kd and log ID50 for the control experiment, day 2 and day 3.

DISCUSSION

The present study shows that during prolonged physical stress there are time dependent alterations in the adrenergic receptors. There is a decrease in the β_2-adrenoceptor number and affinity in both granulocytes and mononuclear cells accompanied by an increase in the ID50 value for adrenaline displacement in the granulocytes. The minimum density (about 50%) was reached after about 2 days (granulocytes) and 4 days (mononuclear cells) of continuous and hard physical activities. The significant correlation between the decreased β_2-adrenoceptor number and the elevated plasma catecholamine levels indicates a mechanism of homologous regulation with receptor internalization as the main cause for this down-regulation. As for the β_2-receptor, the platelet α_2-receptor showed a biphasic response during the course, with a decrease (15%) in the receptor density and affinity on day 2 followed by an increase to about 20 % above control level on day 4. This is well in accordance with other investigators who have shown decreased receptor number and affinity after short term bicycle exercise (Hollister *et al.*, 1981) and increased receptor density and affinity after endurance training (Lockette *et al.*, 1987). In contrast, Lehmann *et al.* (1984, 1986) found reduced Bmax and Kd for the platelet α_2-receptor and increased β_2-receptor density in leucocytes from endurance trained subjects.

The most surprising finding was the retro-regulaton of the α_2- and β_2-receptors from day 3 in spite of persistently high agonist concentrations. This implies a mechanism of heterologous up-regulation. It is interesting to note that this up-regulation of the β_2-receptors on granulocytes as compared to lymphocytes shows a different time course, indicating that different tissues under stress may be regulated differently. This may also be explained by the fact that the granulocytes and the mononuclear cells in blood are replaced at different rates.

Both decreased (FitzGerald, 1981) and increased (Burman *et al.*, 1985) β_2-receptor density has been found after short term exercise. Isoprenaline infusion for one hour increased the β_2-receptor density in peripheral mononuclear cells, whereas continuation of the infusion for 4-6 hours gradually decreased the β_2-receptor density (Tomah and Cryer, 1980). This increase in receptor density was explained by influx of receptor enriched cells into the circulation either from extra-vascular sites or by acute "uncovering" of receptor sites that may have been blocked. This indicates that there is at least a threephasic adrenergic receptor response to exercise as a function of time. If the exercise lasts for some minutes only, there is an increased receptor density. If then the exercise continues for hours and up to 2 days, there is a down-regulation. An extension of the period beyond 2-3 days causes a retroregulation of receptor density in spite of continuous high catecholamine levels.

Salt depletion is known to decrease the number of α_2- and β_2-receptors and may contribute to the decrease observed (Fraser *et al.*, 1981, Mulvihill-Wilson and Pettinger, 1981, Volpe *et al.*, 1982), because the salt intake was reduced from 12 gr normally to 2-3 g during the course (Opstad *et al.*, 1985).

Thyroid hormone excess causes a hyperadrenergic state, with an increased number of β_1- and β_2-adrenergic receptors and a decrease in the α_2-receptor density in the heart. Thyroid deficiency leads to the opposite results. The interconversion of adrenergic receptors may also be regulated by thyroid hormones but in a tissue dependent manner. The reports on the influence of thyroid hormones on the blood cell adrenoceptors are, however, contradictory (Williams *et al.*, 1977, 1979, Smith *et al.*, 1981, Ginsberg *et al.*, 1981, Fox *et al.*, 1985). Thyroid hormones showed a biphasic response during the ranger training course, with an increase during the first day of activities followed by a decrease corresponding to the half life of thyroxine during the rest of the course (Opstad *et al.*, 1984). Alterations in the thyroid hormones therefore can not explain the variation in adrenergic receptor levels in blood cells.

Adrenocorticosteroids induce a different regulation of lymphocyte and granulocyte β_2-receptors. The ß-receptors on granulocytes increased by 40% four hours after cortisone administration, whereas those on lymphocytes were decreased by 40 %. At 24 hours the ß-receptor density was increased by 20 % in both cell types (Davies and Lefkowitz, 1980, 1981). A 100 % increase in β_2-receptors in cultured human lung cells was found after 24 hours incubation with pharmacological doses of hydrocortison (Fraser and Venter, 1980). Since gluco steroids show increased plasma levels and extinguished circadian rhythm during the ranger training course (Opstad *et al*, 1984, Opstad, 1991), the up-regulation may be promoted by them.

There is evidence for sex steroids affecting both α_2- and β_2-adrenergic receptors in a variety of animal tissues. Studies on the relationship between adrenergic effector systems and sex hormones in human blood cells are limited (Rosen *et al.*, 1985). It is therefore uncertain if the 80 % decrease in adrenal steroids during the course may be of significance for the alterations in adrenergic receptors (Opstad *et al.*, 1983).

Previously we have found a strongly increased plasma catecholamine response to a standardized exercise test without similar increases in the blood pressure and pulse rate response (Opstad *et al.*, 1980, Opstad, 1990, 1991). If alterations corresponding to those in leucocytes take place in the cardiac adrenergic receptors, down-regulation of the ß-receptor may be an important mechanism for adrenergic desensitization and reduced working capacity during prolonged stress. The fact that

the catecholamine response to bicycle exercise was similar on day 4 and day 6 in spite of different number of ß-receptors, indicates that there are also other mechanisms leading to desensitization. These may gradually take over when the receptor number normalizes. One such mechanism could be receptor uncoupling (Feldman *et al.*, 1983a, b, Fraser *et al.*, 1981).

The adrenergic responsiveness in vivo is regulated by a complex interaction between target cells and blood borne substances such as hormones and peptides. Desensitization may be a mechanism by which the cells protect themselves against overstimulation.

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PAPER IV

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Adrenaline stimulated cyclic adenosine monophosphate response in leucocytes is reduced after prolonged physical activity combined with sleep and energy deprivation

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Summary. The mechanism for adrenergic desensitisation during physical stress was studied by measuring [^{125}I] cyanopindolol ([^{125}I]CYP) binding sites and the adrenaline stimulated cyclic adenosine monophosphate (cAMP) responses in peripheral blood leucocytes from ten male cadets during a 5-day military training course. The cadets had physical activities around the clock corresponding to a daily energy consumption of about 40,000 kJ but with an intake of only 2,000 kJ, and only 1–3 h of sleep in the 5 days. During the course, the maximal cAMP response to adrenaline stimulation was reduced to about 45% in granulocytes and to 52% in mononuclear cells, and the half maximal response was obtained only at 5--10 times higher adrenaline concentrations than in the control experiment. The binding sites for [^{125}I]-CYP in mononuclear cells increased during the course. However, [^{125}I]-CYP measured not only surface receptors but also intracellular receptors and might even have represented other binding sites. In conclusion, this study showed that decreased cAMP response to adrenergic stimulation would seem to be one of the mechanisms behind adrenergic desensitisation during stress.

Key words: Stress exercise – Fasting – Cyclic adenosine monophosphate – Leucocytes

Introduction

Adrenergic receptors mediate diverse metabolic and neuro-endocrine actions of the catecholamines, adrenaline and noradrenaline. The hormone sensitive adenylate cyclase of mammalian cells detects external hormonal signals and transduces them across the plasma membrane into changes in the rate of cyclic adenosine monophosphate (cAMP) synthesis. Each of these events – detection, transduction, and cAMP synthesis – involves distinct classes of membrane proteins. Receptors, integral membrane proteins with specific binding sites for endogenous ligands and drugs, detect the signal. The ligand-receptor interaction then leads to a stimulation of cAMP synthesis by the catalytic adenylyl cyclase, an integral membrane protein whose enzymatic site faces the cytoplasm. A group of GTP(guanosine triphosphate)-binding proteins has been shown to convey information from the receptor to the catalyst (Lohse et al. 1989, 1990a,b; Fraser and Venter 1990; Collins et al. 1991).

Alterations in leucocyte sensitivity to adrenaline stimulation have been found to take place at different levels such as alteration in the receptor number, receptor uncoupling from the stimulatory G-protein, alteration in adenylyl cyclase activity and finally alterations in the degradation of cAMP by phosphodiesterase (Casperson and Bourne 1987; Hausdorff et al. 1990; Collins et al. 1991; Homcy et al. 1991; Yu et al. 1993). The molecular mechanisms underlying rapid and long-term desensitisation may be different. During short term exercise the number of leucocyte β-receptors have been shown to increase (Burman et al. 1985; Fujii et al. 1993), whereas during prolonged exercise the number of β-receptors decrease during the first 2 days, probably due to a homologous downregulation, followed by a heterologous upregulation when the activities continue for several days (Opstad 1990; Opstad et al. 1994). In the present paper, we have extended our studies on the mechanisms for reduced tissue sensitivity under stress by measuring alterations in the cAMP responses to adrenaline stimulation in leucocytes ex vivo.

In our previous study (Opstad et al. 1994), the use of a β_2-receptor agonist [^{3}H]-hydroxybenzylisoproterenol (HBI) as the radioligand in the receptor binding study may be criticised because the agonist could in itself induce, for example, desensitisation and downregulation of the receptor. We therefore repeated the binding studies with the most used radioligand [^{125}I]-cyanopindolol ([^{125}I]-CYP), which is a β_2-antagonist.

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Methods

Subjects and training course.

The subjects of this investigation were 10 male cadets from the Norwegian Military Academy who participated in a military endurance training course as a part of their training programme. They were aged between 21 and 25 years, and in good mental and physical condition. The subjects gave their informed consent and was free to withdraw from the investigation at any time. The course started on a Sunday afternoon (day 1) and finished on the following Friday (day 6). The cadets were subjected to physical exercise day and night corresponding to 35% of maximal oxygen uptake or a daily energy consumption of about 40,000 kJ for each cadet (Waldum and Huser 1974; Aakvaag et al. 1978). On day 1 the subjects had a normal breakfast and lunch, before they started the activities in the afternoon. During the course they were not given any food except for a cooked chicken for two cadets in the afternoon on day 4. Water intake was not restricted, but in spite of this the participants complained of thirst. The cadets had a loss of body mass of 6–8 kg during the course, of which about 3–4 kg was fat (Rognum et al. 1982), 2 kg was probably glycogen (including glycogen bound water); the remainder was probably muscle protein. There was no organised sleep during the whole training course, but the cadets had very short periods of sleep between activities, totalling about 1–3 h. During the course the weather was fine but with rather low temperatures, particularly at the beginning of the course; 10–15° C by day and minus 5–0° C at night. This resulted in high activity by day and lower activity at night. The course took place during September in a forest area in eastern Norway at about 500-m altitude.

Blood sampling.

Blood was drawn daily between 0600 hours and 0800 hours in the field. The blood was collected into ice-chilled, evacuated 10 ml tubes containing 0.12 ml of 0.34 $mol \times l^{-1}$ ethylenediaminetetraacetic acid (EDTA) K3, which were kept on ice, and immediately transported from the training area to the laboratory, where the leucocytes were separated 5–6 h later.

Cell separation.

A quantity of 5 ml 6% dextran in 0.9% NaCl was mixed with 50 ml of EDTA-blood. After approximately 30 min the erythrocytes had settled and the leucocyte rich layer was transferred into another set of 50-ml tubes. About 12 ml of Lymphoprep (Nycomed) was added to the bottom of the tubes. After centrifugation for 15 min at 600 *g* the mononuclear cells which were collected from the interface between plasma and Lymphoprep were washed twice with 8–10 ml 0.9% NaCl solution and centrifuged at 600 *g* for 15 min after the first wash and for 6–7 min after the second. To remove platelets, the cell button was resuspended again in 6-ml 0.9% NaCl and centrifuged at low speed (70 *g*). The granulocytes which settled during the Lymphoprep separation were resuspended twice in 4-ml 0.83% NH_4Cl for 7 min at room temperature to obtain haemolysis. Finally both mononuclear cells and granulocytes were resuspended in hypotonic 50 $mmol \times l^{-1}$ TRIS buffer containing 10 $mmol \times l^{-1}$ $MgCl_2$ and 30 $mmol^{-1}$ NaCl, pH = 7.4 (Bøyum 1993). Cell numbers were counted in a Hycel Counter-202 (Hycel, Inc., Houston, Texas) and smears for light microscopy differential count were prepared.

cAMP accumulation studies.

Granulocytes or mononuclear leucocytes (10^7 cells $\times ml^{-1}$) in 2.5-ml CMRL-1066 medium (Gibco) with 1.2 $mmol \times l^{-1}$ IBMX (Sigma) were incubated for 20 min at 37° C in a shaking waterbath. After centrifugation at 300 *g* for 5 min at 4° C the supernatant was discarded and the cells were resuspended in 2.5-ml CMRL-1066 with 1.2 $mmol \times l^{-1}$ IBMX, and kept at 4° C. A quantity of 200 ml of the cell suspension containing about two million cells was pipetted into ten Minisorb plastic tubes (3 ml), and 50 ml of a phosphate buffer was added with adrenaline at the following concentrations: 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, $0.3 \cdot 10^{-4}$, 10^{-4}, $0.3 \cdot 10^{-3}$ and 10^{-3} $mol \times l^{-1}$. The cells were incubated for 5 min at 37° C, after which the adrenaline stimulation was stopped by heating the cell suspension for 3 min in a water bath at 97° C. The cell suspension was stored at –80° until the cAMP concentration was analysed.

The cAMP was analysed with RIANEN cAMP[^{125}I] radioimmunoassay kit (cat.no. NEK-033) after extraction with 6% trichloroacetic acid and four washing times in ether. The aqueous phase was lyophilised and the residue was redissolved in phosphate buffer. The recovery was about 90%. Due to the high sensitivity of the assay, the samples were diluted 1:100 before the radio-immunoassay. The intraassay coefficient of variation was 5.4% and the interassay coefficient of variation was 6.2%.

Receptor binding.

Granulocytes and mononuclear cells (five millions) were incubated at 37° C for 60 min with increasing concentrations of [^{125}I]-CYP (81 $TBq \times mmol^{-1}$) (2×10^{-11}, 3×10^{-11}, 6×10^{-11}, 10^{-10}, 3×10^{-10}, 6×10^{-10}, 10^{-9} M) in a total volume of 250 ml of a 50 $mol \times l^{-1}$ TRIS-HCl buffer containing 10 $mmol \times l^{-1}$ $MgCl_2$ and 30 $mmol \times l^{-1}$ NaCl, pH = 7.4. The specific binding was defined as the tracer binding that could be displaced by $5 \cdot 10^{-6}$ $mol \times l^{-1}$ timolol. Bound and free radio-activity were separated by a filter method using a Skatron cell harvester. The dissociation constant (K_d) and maximal binding capacity (B_{max}) for the receptors were calculated by a computer program based on the law of mass action. The parameters were fitted to the nontransformed equilibrium data of the displacer and the [^{125}I]-CYP. Calculation of K_d and B_{max} were based on linear regression of at least seven data points.

Displacement.

Granulocytes and mononuclear cells were resuspended in a hypotonic buffer (50 $mmol \times l^{-1}$ TRIS-HCl, 10 $mmol \times l^{-1}$ $MgCl_2$ and 30 $mmol \times l^{-1}$ NaCl, pH = 7.4) to swell the cells. One million cells were incubated with 100 $pmol \times l^{-1}$ of [^{125}I]-CYP (81 Tbq/$mmol^{-1}$) and increasing concentrations (10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} $mol \times l^{-1}$) of displacer which was adrenaline, noradrenaline, serotonin or timolol. Bound and free radioactivity were separated by a filter method using a Skatron cell harvester. The filters were put into plastic tubes and the radioactivity was counted in a Gamma Master γ-counter for 10 min.

Statistics.

The results showed normal distribution. Analysis of variance for repeated measures (4V, BMDP Statistical Software, Los Angeles) was used with days as the repeated factor, and the Heyn-Feldt correction for departure from sphericity assumption. The Student's *t*-test was used to identify significant alterations. Alterations during the course are referred to the mean of the two controls.

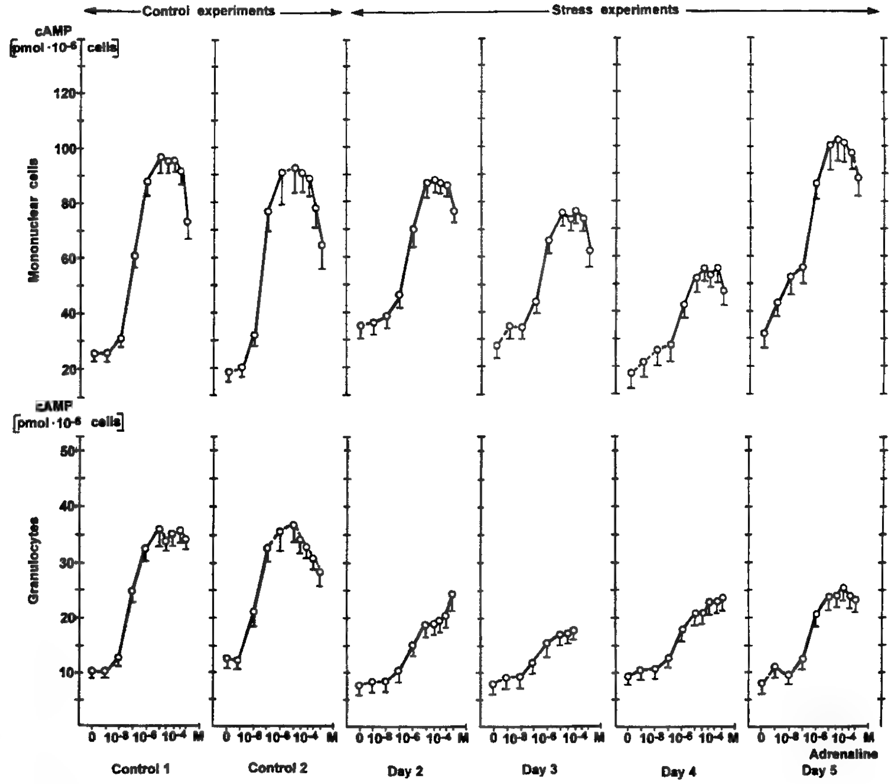

Fig. 1. Cyclic adenosine monophosphate (*cAMP*) response to adrenaline stimulation in human mononuclear cells and granulocytes during a 5-day military training course with heavy physical activities, sleep and energy deficiency. The experiments were performed on days 2–5 and in two control experiments performed while the cadets had normal activities at the training Academy. The results are shown as means and SEM. Time to time variations statistically significant at $P<0.01$ are shown with *thick lines*

Results

The adrenaline stimulated cAMP response was reduced during the course in granulocytes ($F_{2.35,21.19} = 10.19$; $P = 0.0005$) as well as in mononuclear cells ($F_{3.08,27.28} = 3.71$; $P<0.0223$) (Figs 1 and 2). The maximal cAMP response to adrenaline stimulation was reduced in the granulocytes (to 70% on day 2, 43% on day 3, 59% on day 4 and to 67% on day 5) ($F_{8.34,75.02} = 13.35$; $P<0.00005$) and in the lymphocytes (to 73% on day 2, 67% on day 3, 52% on day 4 and 96% on day 5) ($F_{3.85,34.69} = 6.11$; $P = 0.0009$). During the course, half maximal cAMP response to adrenaline stimulation (Fig. 2) was obtained at 4–30 times higher adrenaline concentration in granulocytes ($F_{1.16,10.41} = 7.07$; $P = 0.0190$) and at 4 to 8 times higher concentration in mononuclear cells ($F_{1.41,22.67} = 6.07$; $P = 0.0209$) than in the control experiments.

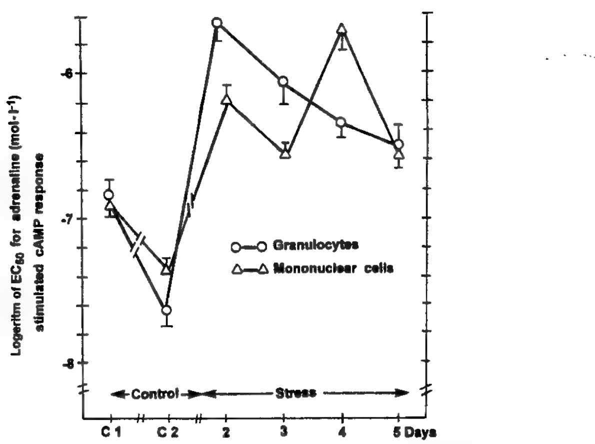

Fig. 2. Changes in effective concentration inducing half maximal capacities response for adrenaline stimulated cyclic adenosine monophosphate (*cAMP*) response in leucocytes during the training course. For details see Fig. 1

Table 1. Alterations in [^{125}I]-cyanopindolol binding [maximal binding capacitis (B_{max}), pmol·10^{-6} cells; and dissociation constant, (K_d), 10^{-10} mol·l^{-1}] in granulocytes (Gran) and mononuclear (Mono) cells during a 5-day military training course

	Ctr 1		Ctr 2		Day 2		Day 3		Day 4		Day 5	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
B_{max}(Gran)	136	16	111	16	170	26	130	26	124	16	152	23
K_d(Gran)	5.5	1.3	4.4	1.0	8.9	1.9	1.3	2.8	6.3	0.8	7.0	1.1
B_{max}(Mono)	340	28	288	15	749	55	610	79	528	45	549	39
K_d (Mono)	5.0	0.5	4.7	0.3	11.1	0.9	20.6	3.3	11.1	1.7	9.7	1.5

The B_{max} of [^{125}I]-CYP binding to mononuclear cells increased two to threefold during the course ($F_{3.17,28.51} = 5.86$; $P = 0.0026$) as did K_d ($F_{2.78,24.98} = 8.46$; $P = 0.0006$) (Table 1). No significant alteration was found for B_{max} in granulocytes, whereas K_d increased during the course ($F_{3.26,29.37} = 3.3$; $P = 0.0309$).

No significant displacement by adrenaline, noradrenaline or serotonin was found for [^{125}I]-CYP binding to granulocytes or to mononuclear cells (data not shown). In contrast, timolol, which is another β-blocker, showed a maximal displacement of [^{125}I]-CYP binding to mononuclear leucocytes and granulocytes of 88% and 80% respectively; inhibitory concentrations giving 50% maximal response were 4.4 (SEM 2.1) $mmol \times l^{-1}$ for mononuclear cells and 5.7 (SEM 3.6) $mmol \times l^{-1}$ for granulocytes.

Discussion

The present study extends the understanding we have gained of the mechanisms of adrenergic desensitisation which takes place during prolonged strenuous physical stress (Opstad 1990). Previously, using HBI as radioligand, a 50% decrease has been observed in the number of β-receptors, together with a small increase in K_d, in both granulocytes and mononuclear cells (Opstad et al. 1994). This decrease corresponded to the decrement found in the maximal cAMP response to adrenaline stimulation. The reduced sensitivity to adrenaline stimulation showed that in addition to the previously demonstrated reduced number of β-receptors on the leucocyte surface, this downregulation also resulted in reduced cAMP accumulation following adrenergic stimulation.

It has been suggested that the reduced cAMP accumulation might have several explanations, such as phosphorylation of the receptor by cAMP-dependent kinase, phosphorylation by the specific agonist dependent β adrenergic receptor kinase, sequestration of the receptor away from the cell surface and into a membrane associated compartment, and decreased adenylate cyclase activity (Lohse et al. 1990a,b; Yu et al. 1993). The detailed mechanism for the decreased cAMP response to adrenaline stimulation during the present course is unknown. There is, however, good reason to think that several of the mentioned mechanisms might be involved.

In a previous investigation demonstrating downregulation and desensitisation of leucocyte β-receptors (Opstad et al. 1994), we have used an agonist: HBI, to evaluate B_{max} and K_d. However, an objection to the use of an agonist as radioligand is that the ligand itself might stimulate the receptor and hence change the number and affinity of the adrenergic receptors. However, with the short incubation time used (15 min), the internalisation was probably minimal. To avoid this possible problem we wanted, in the present study, to repeat the experiment using the antagonist [^{125}I]-CYP as radioligand. However, since [^{125}I]CYP is lipophilic it also passes through the membrane and binds to intracellular receptors. In addition, it has been reported that CYP may also bind to unspecific binding sites (Sandnes et al. 1987a,b; Fujii et al. 1993), in spite of the fact that 90% was displaced in a competition binding with timolol. The increase in CYP binding to mononuclear cells during the course therefore expresses a mixture of binding to both extra and intracellular binding sites. If the intracellular binding of CYP represents the β-receptor, the present results indicate an increased β-receptor density intracellularly in the mononuclear cells during the course. This might explain the differences that have been observed in β-receptor changes during short term and long-term exercise (FitzGerald et al. 1981; Burman et al. 1985; Frey et al. 1989; Maki 1989; Graafsma et al. 1990).

The increase in β-receptor density which has been found to take place during short-term exercise cannot be explained by increased synthesis of new receptors since this normally takes several hours. However, it might be explained by unmasking of already existing receptors or mobilisation of other groups of mononuclear cells such as monocytes or more active lymphocytes which normally are located at the borders of the vessels. During the training course all these factors might have contributed to the present results; including synthesis of new intracellular receptors, internalisation and uncoupling of the receptor by phosphorylation. A twofold increase in monocytes and a 50% decrease in lymphocytes during the course may also explain some of these alterations (Bøyum et al. 1992).

One might speculate whether the described alterations in blood cell β-receptors or adrenergic sensitivity are representative for other tissues such as the heart. Previously we have concluded that the β-receptor downregulation during the course is multifactorial but with a strong homologous component. For homologous regulation, it has been shown that the β-receptors in leucocytes and heart show similar responses (Brodde et al. 1989; Sbirrazzuoli and Lapalus 1989). We might, therefore, conclude that the decreased blood pressure and pulse rate response to increased plasma concentrations of noradrenaline and adrenaline are due to a downregulation of β-receptors as well as decreased cAMP response to adrenaline stimulation.

In conclusion, the present study showed that the previously demonstrated decrease in the number of β-receptors on granulocytes and mononuclear cells during prolonged physical stress, with sleep and energy deficiency, also resulted in a decreased cAMP response to adrenaline stimulation. The [^{125}I]-CYP binding sites were increased in mononuclear leucocytes. This did not, however, result in stimulation of adenylate cyclase in response to adrenergic stimulation.

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PAPER V

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Androgenic Hormones during Prolonged Physical Stress, Sleep, and Energy Deficiency

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ABSTRACT. Androgenic hormones were investigated during two separate 5-day military endurance training courses, with physical activities around the clock corresponding to a daily energy consumption of about 40,000 kilojoules, but with an intake of only 2,000 kilojoules. Altogether, the cadets slept for 1–3 h in the 5 days. Eleven male cadets participated in course I, and 10 in course II.

Plasma levels of testosterone, free testosterone, dehydroepiandrosterone, 17α-hydroxyprogesterone, and androstenedione decreased by 60–80% during the course. In contrast, plasma cortisol, aldosterone, progesterone, and dehydroepiandrosterone sulfate increased.

LH, FSH, and ACTH decreased to about 50–80% of precourse levels. Weak correlations between plasma levels of hypophyseal and levels of adrenal and testicular hormones indicate a multifactorial regulation.

In conclusion, both adrenal and testicular androgens decrease during prolonged physical strain combined with energy and sleep deficiency. (*J Clin Endocrinol Metab* **74:** 1176–1183, 1992)

ALTHOUGH not strictly necessary for life itself, androgens are essential for sexual behaviour and function, initiative and aggressive behavior, muscle strength, and protein synthesis. These hormones are also (mis)used by athletes for the purpose of increasing muscle mass and strength.

Most studies show an increase of 10–30% in plasma testosterone during short term exercise (1–6) due to hemoconcentration (7), decreased degradation (8), and probably also increased testicular secretion. Catecholamine infusion into the spermatic artery increases testosterone secretion (9), and β-blockers inhibit the testosterone increase during exercise (10). The increased plasma LH and FSH during short term exercise may, together with the increased catecholamines, explain the increased plasma levels of testosterone during short term exercise.

Under prolonged physical stress, such as bicycle competition lasting for several days (11), psychological stress in military operations (12, 13), and surgery and anesthesia (14, 15), the plasma levels of androgens decrease. During physical stress, with sleep and energy deprivation lasting for several days, testosterone, dehydrotestosterone, and androstenedione decrease by 70–90% due mainly to the effects of physical exercise (16, 17).

During prolonged physical stress combined with lack of food, metabolism is directed at energy mobilization from fat and proteins. Thus, all catabolic hormones, such as glucocorticoids, increase, whereas anabolic hormones, such as testicular androgens, decrease. Like cortisol and aldosterone, adrenal androgens are stimulated by ACTH, but unaffected by LH/FSH (18). Since the adrenal androgens are anabolic hormones, we should expect them to decrease. On the other side, since they are adrenal steroids like cortisol and regulated by ACTH, we should expect them to increase. We, therefore, wished to know if the adrenal androgen responses to prolonged exercise are similar to those of other adrenal steroids or testicular androgens, or if they behave quite independently. We have also studied their regulation by hypophyseal hormones.

Materials and Methods

Subjects and training course

The subjects for this investigation were 21 male cadets from the Norwegian Military Academy who took part in 1 of 2 military endurance training courses as part of their training program. Eleven of these cadets participated in course I, and 10 in course II. They were between 21–25 yr old and in good mental and physical condition. The courses started on a Sunday afternoon (day 1) and finished on the following Friday (day 6). The cadets were subjected to heavy physical exercise day and night, corresponding to 35% of VO_2 max or a daily energy consumption of about 40, 000 kilojoules (kJ) for each cadet. On day 1, the subjects had a normal breakfast and lunch before they started the activities in the afternoon. The food contained about 5000 kJ the second day, 3000 kJ on day 3, a cooked

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chicken (with extra salt during course I because of high ambient temperature) in the afternoon on day 4, and only some bread (2000 kJ) on day 5. Water intake was unlimited, but in spite of this, the participants complained of thirst. They had a 3- to 4-kg reduction in body fat during the course. There was no organized sleep during the whole training course, but the cadets had very short periods of sleep between activities, totalling about 1–3 h. During course I the weather was fine, with temperatures between 25–32 C by day and 15–20 C at night. This resulted in reduced activity during the daytime and higher activity levels during the night. In contrast, the weather during course II was rainy, with temperatures between 10–20 C during the daytime, and snow and temperatures below 0 C during the night, resulting in high activity by day and minimal activity at night. The course took place during September in a forest area in the eastern Norway at about 500 m altitude.

Blood sampling

Blood was drawn daily between 0600–0800 h in the field. The blood was collected into ice-chilled evacuated 10-mL tubes containing 0.12 mL 0.34 M EDTA K_3 and 5000 kallikrein inhibitor units aprotinin. These were stored on ice and centrifuged within 60 min in a refrigerated centrifuge, and the plasma was frozen immediately to −80 C on dry ice and kept at that temperature until analyzed.

Chemical analysis

Blood glucose was analyzed using Beckman's Glucose Analyser 2 with an oxygen-sensitive electrode. The hormones were analyzed by radioimmunological methods (RIA). Intraassay (first) and interassay (second) coefficients of variation are given in parentheses. Since all samples from each course were analyzed in one assay and with the same standards, the interassay variation has relevance only in the comparison between the two courses. Cortisol (4.4% and 8.4%), progesterone (6.8% and 15%), 17α-hydroxyprogesterone (5.8% and 6.3%), dehydroepiandrosterone (DHEA; 7.7% and 13%), aldosterone (5.4% and 12%), testosterone (8% and 11%), free testosterone (5.9% and 6.6%), and estradiol (6.5% and 9.4%) were analyzed with Coat-a-Count kits, and LH (3.6% and 7.6%) and FSH (3.2% and 6.5%; course II) were analyzed with a double antibody kit from Diagnostic Products Corp. (DPC; Los Angeles, CA). In course I, LH (4.5% and 6.9%) and FSH (3.7% and 7.5%) were analyzed using Quik RIA kits (double antibody kits) from Pacific Biotech (CA). In both courses, LH (2.6% and 6.7%) was also analyzed with an IRMA-Count kit from DPC. The samples from course I was analyzed for DHEA sulfate (DHEAS) with a kit from Diagnostic Systems Laboratories (DSL; TX; 5.6% and 8.4%), whereas the samples from course II were analyzed with a kit from DPC (4.5% and 14.5%). Androstenedione (4.6% and 9.7%) was analyzed with solid phase RIA kits from DSL.

Statistics

The results are presented as the average ± SEM. Analysis of variance for repeated measures (BMDP, 4V) was used, with days as the repeated factor. The *t* test was used to identify overall significant alterations. Correlation coefficients were calculated according to the method of Kendall and Spearman (BMDP, 3S). Stepwise regression was used to analyze the relationships between hormones (BMDP, 2R).

Results

All hormones were measured in plasma.

Testosterone

Testosterone (nanomoles per L; Fig. 1) decreased in course I from 23.1 ± 3.9 to 5.3 ± 0.7 ($F_{2.09,20.92} = 12.45$; $P = 0.0002$). During course II, the levels decreased from 25.9 ± 1.9 to 6.0 ± 0.4 ($F_{5.52,49.72} = 49.39$; $P < 0.00005$).

Free testosterone

Free testosterone (picomoles per L; Fig. 1) decreased during course I from 137 ± 12 to 28 ± 3 ($F_{1.83,18.28} = 45.36$; $P < 0.00005$) and from 181 ± 8 to 41 ± 3 ($F_{5.72,51.45} = 78.30$; $P < 0.00005$) during course II.

Estradiol

Estradiol (nanomoles per L; Fig. 1) increased during course I from 128 ± 8 to a maximum of 158 ± 11 on day 3, followed by a decrease to 94 ± 9 on day 6 ($F_{3.02,30.16} = 6.75$; $P = 0.0013$). During course II no significant alterations were found.

Androstenedione

Androstenedione (nanomoles per L; Fig. 2) showed a gradual decrease from 9.7 ± 0.7 at the start to 3.9 ± 0.3 at the end of course I ($F_{4.61,46.07} = 34.74$; $P < 0.00005$). During course II plasma levels decreased from 8.8 ± 0.6 to 4.9 ± 0.5 ($F_{5.47,49.27} = 6.84$; $P = 0.00005$).

17α-Hydroxyprogesterone

17α-Hydroxyprogesterone (nanomoles per L; Fig. 2) decreased during course I from 4.6 ± 0.7 to 1.3 ± 0.2 on day 3 ($F_{1.79,17.86} = 18.66$; $P = 0.0001$). During course II the levels decreased from 5.2 ± 0.5 to 2.2 ± 0.4 by day 4 ($F_{6.78,60.99} = 15.89$; $P < 0.00005$).

DHEA

DHEA (nanomoles per L; Fig. 2) decreased gradually from 27.6 ± 2.9 to 6.2 ± 0.6 at the end of the course ($F_{2.07,20.70} = 25.88$; $P < 0.00005$). During course II plasma DHEA decreased from 24.3 ± 2.4 to a minimum of 10.1 ± 1.3 on day 4 ($F_{4.2,37.79} = 9.68$; $P < 0.00005$).

DHEAS

DHEAS (micromoles per L; Fig. 3) increased from 8.8 ± 0.6 to a plateau of 11.5 ± 0.5 on day 3 ($F_{3.26,32.65} =$

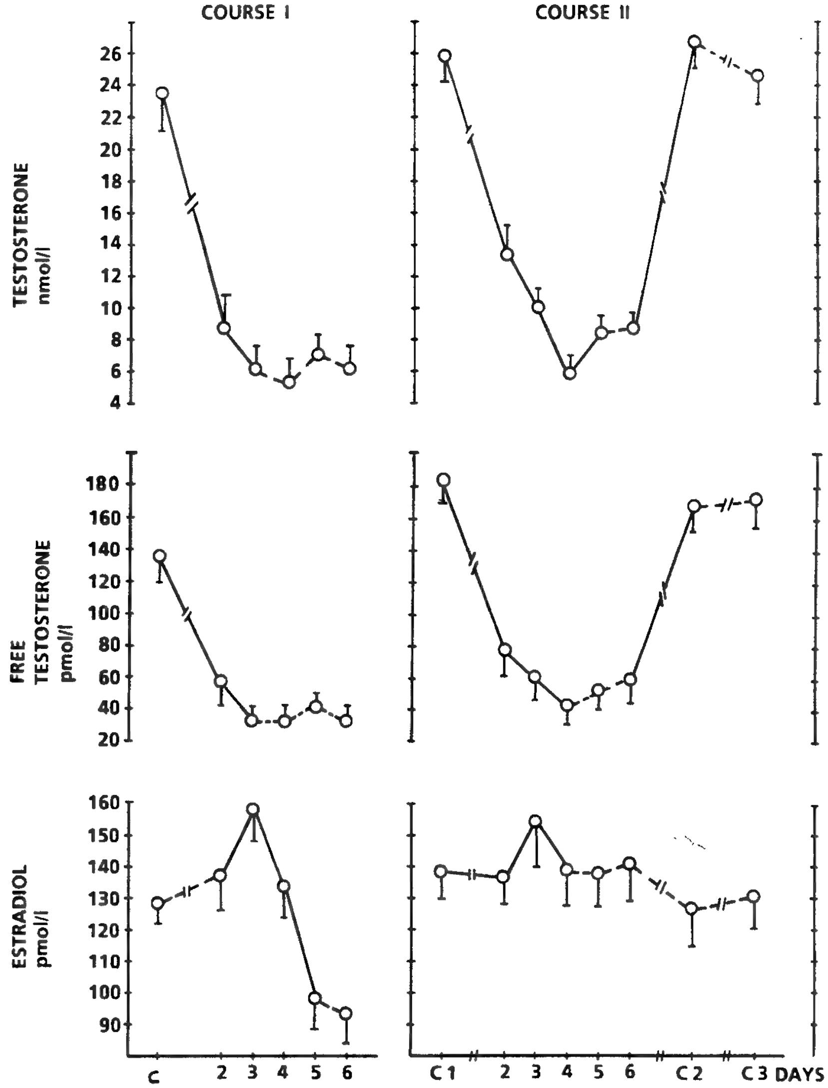

FIG. 1. Plasma total and free testosterone and estradiol in 11 and 10 cadets during 2 5-day military training courses with heavy physical activities, and sleep and energy deficiencies. The control samples C and C1 were obtained 1 week before the course, and C2 and C3 were obtained after 2 months of normal training. Blood samples were collected daily between 0600–0800 h. Variations significant at $P < 0.05$ are indicated by *continuous lines*. The results are presented as the mean ± SEM.

13.95; $P < 0.00005$). During course II the levels increased gradually from 9.5 ± 1.1 to 15.4 ± 1.6 on day 2 and to a maximum level of 21.3 ± 2.9 on day 6 ($F_{2.07,18.63} = 31.16$; $P < 0.00005$).

Progesterone (nanomoles per L; Fig. 3) increased from 2.2 ± 0.2 to 4.2 ± 0.7 on day 2 ($F_{3.66,36.62} = 9.99$; $P < 0.00005$). From then on, the plasma level of progesterone decreased to below the precourse level by days 5 and 6. Also during course II a significant increase was seen in plasma progesterone from 2.14 ± 0.07 to 2.60 ± 0.21 ($F_{3.92,35.32} = 2.92$; $P = 0.0356$).

Cortisol

Cortisol (nanomoles per L; Fig. 3) increased from 542 ± 40 to 860 ± 62 on day 3 ($F_{5,50} = 6.34$; $P = 0.0001$). From days 3–6 a gradual decrease was seen to precourse levels by the end of the course. During course II plasma cortisol increased from 550 ± 44 to a maximum of 698 ± 49 on day 4 ($F_{7,63} = 6.55$; $P < 0.00005$).

Aldosterone

Aldosterone (nanomoles per L; Fig. 3) increased from 0.49 ± 0.08 to 2.02 ± 0.26 on day 3 ($F_{3.46,34.61} = 16.93$; $P < 0.00005$). From days 4–6 a significant decrease was found, probably because of extra salt and reduced physical activity due to hot weather. During course II the plasma level of aldosterone increased from 0.48 ± 0.05

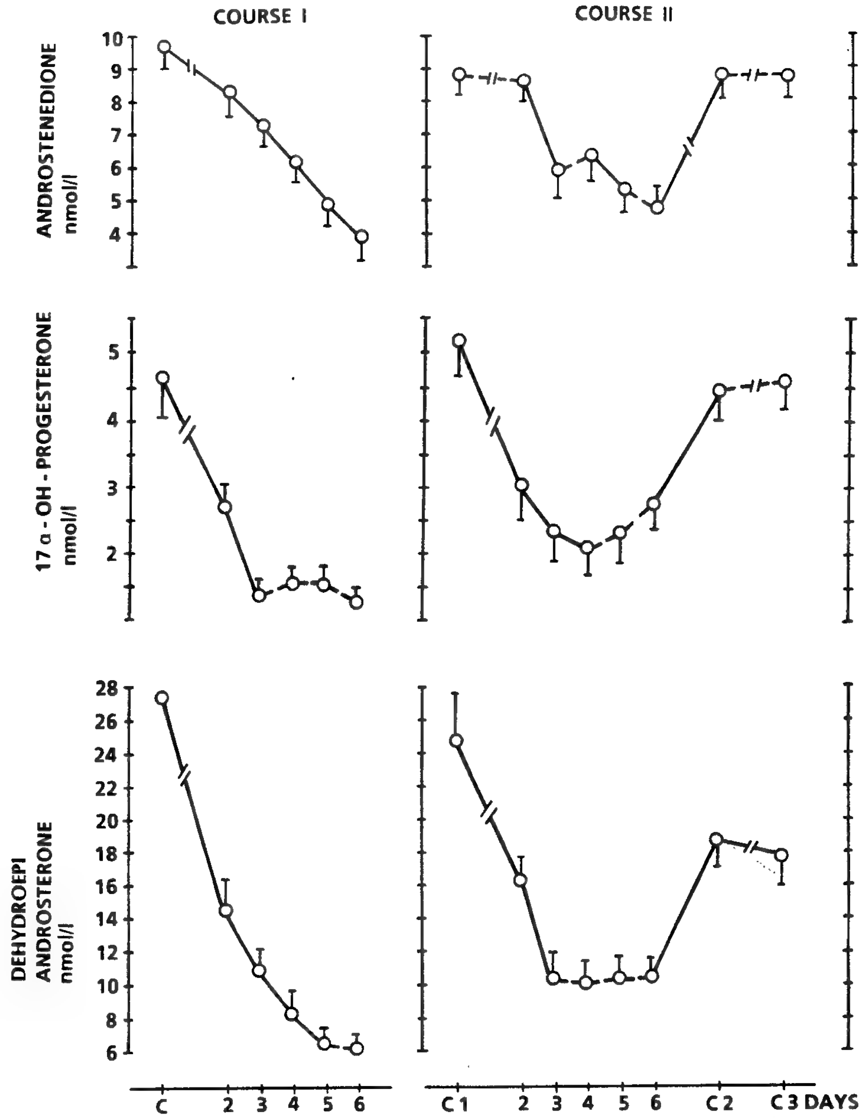

FIG. 2. Plasma levels of androstenedione, 17α-hydroxyprogesterone, and DHEA during the training courses. For details, see Fig. 1.

to 1.79 ± 0.22 on day 4 ($F_{4.86,43.77}$ = 20.25; $P < 0.00005$). No significant alterations were found from days 4–6.

ACTH

ACTH (picomoles per L; Fig. 4) decreased gradually during course II from 5.2 ± 0.6 to 4.0 ± 0.3 on day 6 ($F_{7,63}$ = 5.28; $P = 0.0001$).

LH

LH (international units per L Seconnd International Reference Preparation of human menopausal gonadotropin; Fig. 4). During course I LH decreased from 3.35 ± 0.31 to 2.00 ± 0.07 (immunoradiometric assay kit) on day 2 ($F_{3.87,38.7}$ = 6.89; P = .0003). Analyzed with the Quik RIA kit, plasma LH decreased from 6.84 ± 0.42 to 4.70 ± 0.36 on day 2 ($F_{4.72,47.24}$ = 5.37; $P = 0.0007$). During course II plasma LH, using the immunoradiometric assay kit decreased from 2.58 ± 0.23 to 1.75 ± 0.44 on day 3. An increase was found during the rest of the course to 3.20 ± 0.54 on day 6 ($F_{4.3,38.74}$ = 2.71; $P = 0.0404$). When analyzed using a double antibody kit (DPC), the plasma levels decreased from 6.63 ± 0.66 to 4.92 ± 0.80 on day 2 and further to 3.88 ± 0.92 on day 3. From day 3 the levels increased gradually to precourse levels on day 6 ($F_{4.41,39.71}$ = 3.62; $P = 0.0110$).

FSH

FSH (international units per L Second International Reference Preparation of human menopausal gonadotro-

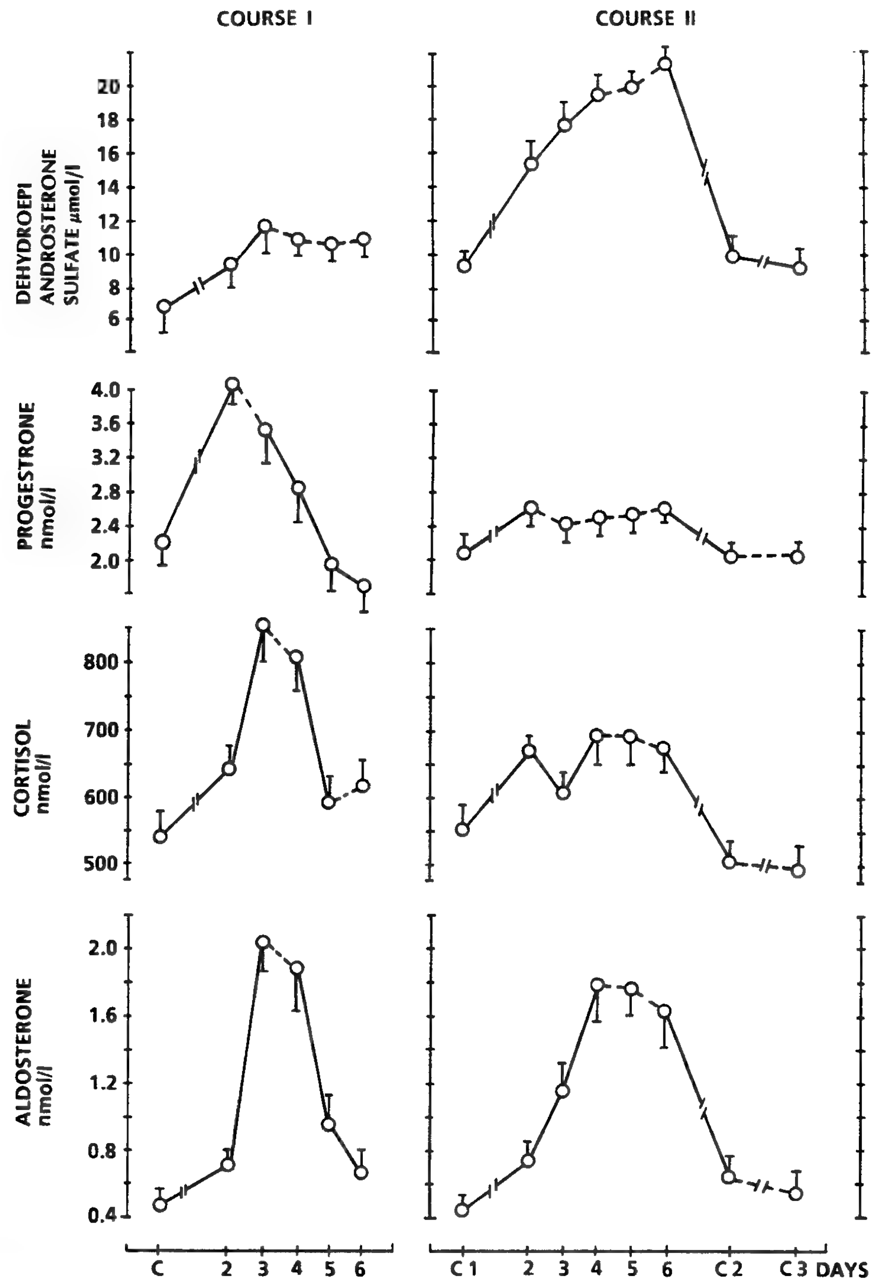

FIG. 3. Plasma levels of DHEAS, progesterone, cortisol, and aldosterone. For details, see Fig. 1.

pin; Fig. 4) decreased gradually from 6.94 ± 1.38 to 4.10 ± 0.89 during course I (Quik RIA; $F_{2.26,\ 22.63} = 3.83$; $P = 0.0326$). During course II plasma FSH decreased significantly from 6.94 ± 0.56 to 5.65 ± 0.68 on day 4 (DPC; $F_{2.02,18.21} = 2.55$; $P = 0.1051$).

Glucose

Glucose (millimoles per L) decreased from 4.6 ± 0.2 to 3.9 ± 0.2 at the end of course I ($F_{3.92,39.25} = 3.88$; $P = 0.0099$). During course II plasma glucose decreased from 4.85 ± 0.08 to 4.12 ± 0.14 on day 5 ($F_{4.25,38.28} = 11.96$; $P < 0.00005$).

Beard growth

Beard growth was evaluated by the cadets. On day 1 the growth was only slighty decreased to about 70–80% of normal growth. On day 2 the growth was reduced to

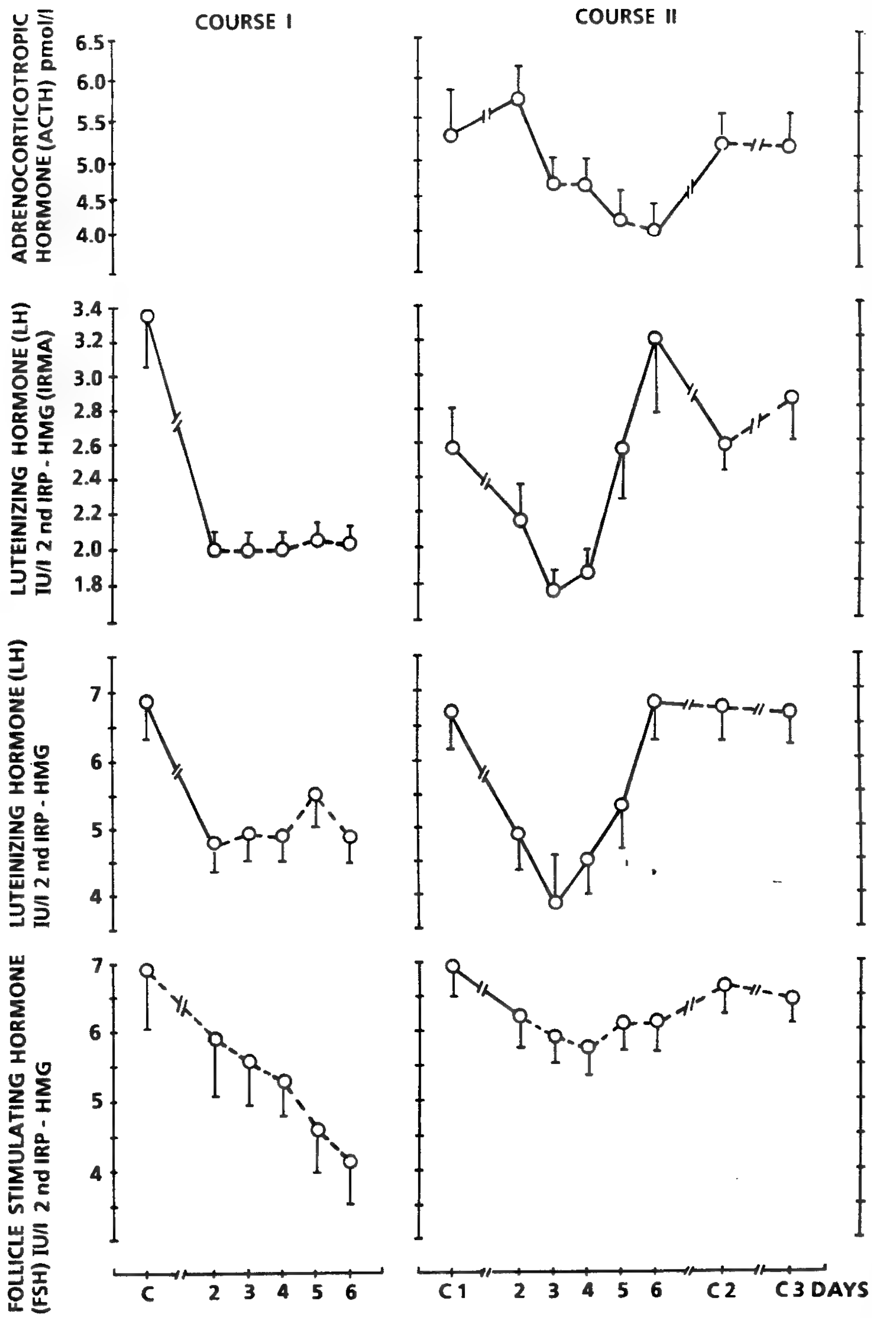

FIG. 4. Plasma levels of ACTH, LH (analyzed by the immumoradiometric assay method from DPC and the Quik RiA kit from Pacific Biotech), and FSH during 5 days of strenuous military activities. For details, see Fig. 1.

30–50% of normal, and for the rest of the course almost no growth was observed.

Correlations

A positive correlation was found between the percent alterations in free and total plasma testosterone (r = 0.65; $P < 0.02$). Significant correlations were also found between the results obtained with the two kits for analysis of plasma LH (r = 0.81; $P < 0.01$) and between LH and FSH (r = 0.69; $P < 0.02$). A stepwise regression analysis with adrenal androgens as dependent variable and cortisol as marker for adrenal secretion and testosterone for testicular secretion showed no significant relationships.

Discussion

The present study shows that there is a decrease in all androgen hormones, testicular as well as adrenal, after hours and days of continuous physical exercise.

Plasma testosterone shows a biphasic response to exercise, with an increase during the first minutes (1–6), followed by a decrease if the duration of exercise extends to hours or days (16, 17). The parallel alterations for free and total testosterone show that there is no alteration in the protein binding of testosterone during prolonged physical stress. The increased plasma cortisol and catecholamine levels during the courses may have contributed to the observed decrease in plasma testosterone (9, 19, 20).

Normally, only 10–25% of plasma DHEA, 25–30% of androstenedione, and 10% of 17α-hydroxyprogesterone derive from direct testicular secretion (21). A total halt in all testosterone production would not be enough to explain the decrease in the plasma androgen levels observed. Therefore, there has to be a decrease in both testicular and adrenal androgen secretion.

Divergence between the adrenal androgens and the glucocorticoids has been described as a function of age in the recovery after treatment with glucocorticoids, during surgery, and after burn injuries (reviewed in Ref. 21). This work shows that this divergence is also present during prolonged exhausting physical stress, in that the androgens decrease, whereas cortisol, aldosterone, and DHEAS increase. The increased DHEAS may be explained by stimulation of the steroid sulfokinase and/or inhibition of the steroid sulfatase in both the adrenals and peripheral tissues acting upon circulating DHEA. Since 80–90% of DHEA and 90–98% of DHEAS are thought to derive from direct adrenal secretion, it is reasonable to suppose that the difference between DHEA and its sulfate form may be explained by different adrenal secretion rates of the two hormones (21, 22). Rather low intensity exercise during the courses suggests only a minimal effect on hepatic circulation and, thus, hormonal clearance. There is no reason to suppose that the hormonal clearance is affected by impaired liver function, since the liver enzymes and galactose test show normal values. With only small variations in total plasma proteins, albumin, and plasma volume, there is no reason to believe that the hormonal clearance is affected by dehydration (23).

The increased plasma level of DHEAS did not compensate biologically for the decreased levels of the nonsulfated forms; for example, there was a strong decrease in beard growth, which during the 5-day course was reduced to the equivalent of 1–2 days of normal growth (24). The cadets also had other clinical symptoms of hypogonadism, such as lack of initiative and defensive behavior, with rather low and changed aggressiveness (25).

ACTH, LH, and FSH are known to regulate more or less all steps in steroid synthesis, but they particularly affect the availability of cholesterol for the side-chain cleavage enzyme in the mitochondria (18, 26). The decrease in plasma LH and FSH may explain the decrease in testicular androgens during the test course. The decrease in ACTH could explain the decrease in adrenal androgens, but is inconsistent with the increase in cortisol. However, an inhibition of C_{17-20}-desmolase activity would explain the decrease in adrenal androgens combined with the increase in cortisol and aldosterone.

To avoid the effect of acute physical exercise on plasma levels of hormones, the cadets had only light physical activity for 1–2 h before blood sampling. This may, however, imply that the subjects were in a state of recovery. For ACTH, with a short half-life, it may even be that the negative feedback from elevated levels of glucocorticoids in the "recovery phase" may explain the decreased levels. Also, the increased LH values at the end of the course may be due to a faster recovery for these hormones than for testosterone. The fact that the plasma levels reflect mainly the instantaneous and not the integrated levels of hormones may also explain the rather weak correlations between the hypophyseal hormones and the steroids. The fact that no significant relationships were found in a regression analysis using cortisol as an index for adrenal secretion and testosterone as an index for testicular secretion indicates that adrenal androgens are regulated independently from cortisol and testosterone.

The decreased plasma aldosterone, cortisol, estradiol, and progesterone levels at the end of course I are probably explained by reduced physical daytime activity and extra NaCl because of the hot weather. Physical activity was also reduced during the night at the end of course II because of snow and rain. Plasma aldosterone and cortisol have been shown to increase during heat exposure (27); this might explain the higher levels in the middle of course I compared to those in course II. The increased aldosterone levels are in accordance with previous results showing that it is mainly regulated by the renin-angiotensin system and the intake of NaCl (28).

In previous experiments no significant alterations were found for plasma estradiol during the first 2 days of activities, followed by a decrease by the end of the course (16). In the present study a small significant increase was found after 1 day, followed by a decrease in course I, but not in course II.

In conclusion, the present paper shows that there is a decrease in all androgenic steroids, except DHEAS, independent of whether they originate from the testes or the adrenals.

Acknowledgments

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PAPER VI

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The hypothalamo-pituitary regulation of androgen secretion in young men after prolonged physical stress combined with energy and sleep deprivation

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During a five days' military training course for male cadets with hard physical activity day and night and almost no sleep or food, a decrease was found in LH, FSH, PRL and TSH. A decrease was also found in testosterone, dihydrotestosterone (DHT), androstenedione, dehydroepiandrosterone and 17α-OH progesterone, whereas dehydroepiandrosterone-sulfate increased twofold. The LH and FSH responses to GnRH intravenously were increased at the end of the course. This demonstrates enhanced pituitary reserves of gonadotropin, or, alternatively, increased sensitivity to GnRH stimulation and may be due to decreased hypothalamic secretion of GnRH during the course. The decreased DHT and testosterone levels were almost normalized after HCG stimulation, indicating a gonadotropin regulated decrease in testosterone secretion during the course. In spite of fairly weak correlation between the alteration in gonadotropins and androgens it is concluded that there is a major regulation of testicular androgen secretion during prolonged stress by the hypothalamo-pituitary axis.

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During physical exercise plasma androgens and gonadotropins show a biphasic response, with a moderate increase when the exercise lasts for minutes followed by a decrease when it lasts for hours or days (1, 2). During continuous military operations lasting for several days, the 90% decrease found in plasma levels of free and total testosterone are due more to continuous physical stress and less to sleep deprivation, whereas energy deprivation has no significant effect (3–5). Also dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), estradiol (E_2), 17α-OH progesterone and androstenedione decrease during prolonged physical stress (5).

The role of gonadotropin-releasing hormone (GnRH) for the alterations in gonadotropins during short- or long-term exercise has not been investigated. GnRH is produced in the preoptico-suprachiasmatic anterior hypothalamus and transported by portal circulation to the anterior hypophysis where it stimulates the release of gonadotropins. In spite of the fact that GnRH-like peptides have also been isolated from the rat testis, and that such peptides may be directly involved in the peripheral nervous regulation of both steroidogenesis and spermatogenesis, GnRH cannot be measured in peripheral blood (6–13).

In the work reported here, the hypothalamo-hypophyseal regulation of the androgenic steroids during prolonged physical stress, combined with sleep and energy deprivation, has been studied by measuring the plasma levels of LH, FSH, prolactin and testicular and adrenal androgens, and responses to GnRH and HCG stimulation.

Materials and methods

Subjects and training course

The subjects were 10 male cadets of the Norwegian Military Academy participating in a military endurance training course (Course I) as part of their training program. Another group of nine cadets from a previous year (Course II) participated in the HCG study. All subjects were between 21 and 26 years of age and in good physical and mental condition. The study was approved by the Norwegian Joint Medical Service. The course started on a Sunday afternoon (Day 1) and finished on the following Friday afternoon (Day 6). The subjects were exposed to continuous physical (infantry) activities previously shown to correspond to 35% of their maximal oxygen uptake or about 40 000 kJ·24 h^{-1} $cadet^{-1}$. On Day 1 before the start of the course the cadets had a normal breakfast and lunch. Energy intake was about 5000 kJ on the second day, 3000 kJ on Day 3, a cooked chicken in the afternoon on Day 4 and only some bread (2000 kJ) on Day 5. Water intake was unlimited, but in spite of this the cadets complained of thirst. They each lost 4 kg of fat during the course. No normal sleep was allowed

but the cadets had short periods of sleep between activities, estimated to be a total of 1–3 h during the whole course. The courses took place in a forested area in the eastern part of Norway, Course I at the end of August and Course II in June. During both courses the weather was fine, cool at night (10–15°C) and warm during the day (25–30°C in Course I and 20–25°C in Course II). Although the program was the same for all courses, there were some restrictions on the physical activities in Course I because of the high day-time temperatures.

GnRH-test and blood sampling

The GnRH test was performed on Day 5 and in a control experiment two months after the course had finished. The test was performed between 07.00 and 08.00. The GnRH test was: 0.1 mg of GnRH (Lutrefact, Hoechst AG) injected through an indwelling Veneflon® cannula in the antecubital vein for 1 min. The blood samples were taken according to the following time schedule: 15 min and 0 min before the GnRH injection and then 10, 20, 30, 45 and 60 min after the GnRH injection. Twenty ml of blood was drawn for each sample through the Veneflon® cannula into ice-chilled evacuated tubes containing 0.12 ml 0.34 mol/l EDTA K3 and 5000 KIU aprotinin. The blood was kept on ice until centrifuged in a refrigerated centrifuge; the plasma was frozen on dry ice and kept frozen at −80°C until analysed.

HCG test

Blood samples for determination of testosterone and DHT were taken before and 3 h after an intramuscular (vastus lateralis) injection of HCG (Physex 3000 IU, Leo). The control experiment was performed two months after the course, and the stress experiment on the last day of the course (Day 6). Both tests were performed in the morning between 08.00 and 11.00.

Chemical analysis

The analyses were run in the same assay and with the same standards. The intra-assay coefficients of variation (%) calculated on the basis of the present results are given in parentheses. LH (10.7) was analysed with IRMA kits from the Diagnostic Products Corporation (DPC), CA. FSH (9.7) and testosterone (9.6) with Maia kits, both from Serono Diagnostics. PRL (8.6) and TSH (2.7) were analysed with double antibody RIA kits, and estradiol (10.3), progesterone (8.5), 17α-OH progesterone (8.7), DHEA (10.1) and cortisol (4.5) with "Coat-a-Count" kits from DPC. DHEA-S (6.5) and androstenedione (4.6) were analysed with RIA kits from Diagnostic Systems Laboratories Inc. Dihydrotestosterone (11.2) and testosterone (9.2) were analysed for the Course II samples using a kit from Amersham, UK.

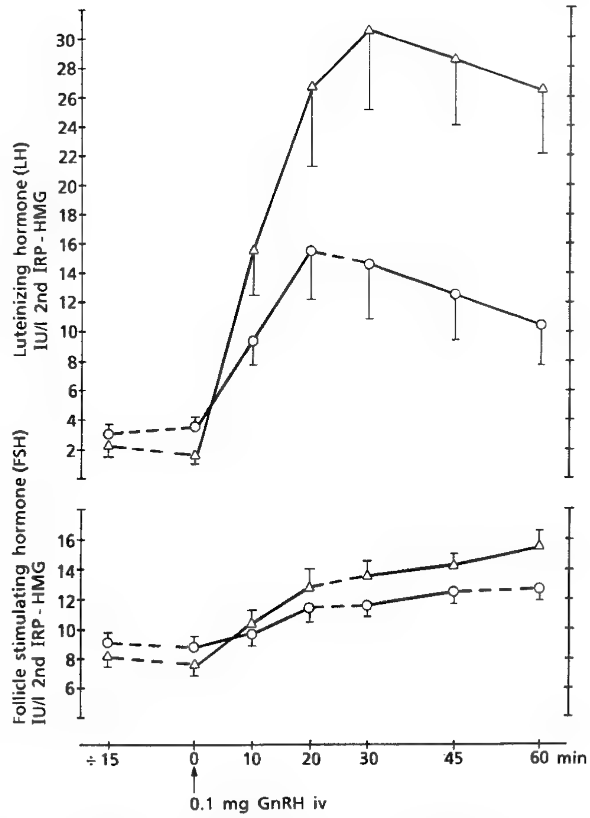

Fig. 1. Alterations in luteinizing hormone (LH) and follicle stimulating hormone (FSH) responses to a bolus intravenous injection of 0.1 mg gonadotropin releasing hormone (GnRH) in young military male cadets after four days of sleep and energy deficiency, with continuous hard physical activity (△– –△). The control experiment (○– –○) was performed two to three months after the course. The results are presented as means ± SEM. Sample to sample variations statistically significant at $p<0.01$ are shown by thick lines and not significant variations by dotted lines.

Statistics

The results are presented as means ± SEM. Sample to sample variations significant at $p<0.01$ are indicated by thick lines. An overall analysis of variance for repeated measurements (BMDP, program: 4V) was used to test significant effects of GnRH stimulation, and possible alterations during stress. Time was used as the repeated factor. The *t*-test was used to identify significant differences and for the HCG results. Spearman correlation coefficients were calculated using BMDP, 3S.

Results

The pituitary hormones LH, FSH, PRL and TSH all decreased during the course (Figs. 1 and 2, Table 1), as did the testicular androgens, dihydrotestosterone and testosterone (Fig. 3, Tables 1 and 2), and the adrenal androgens, 17α-OH progesterone, androstenedione, pro-

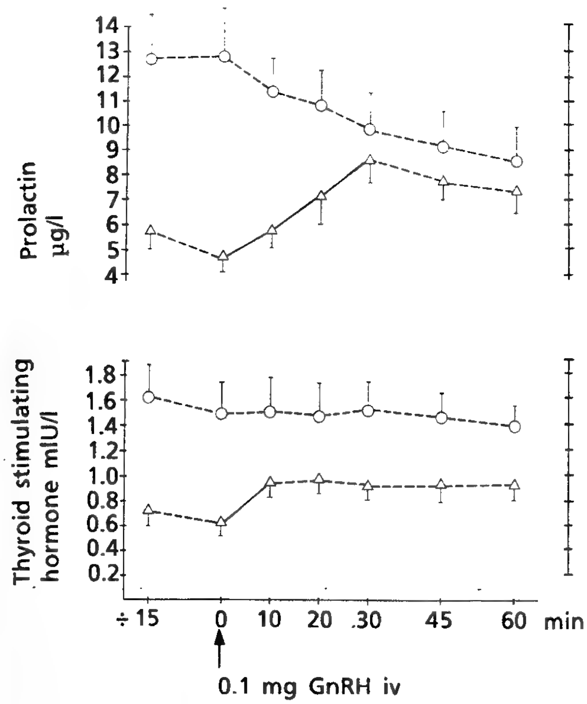

Fig. 2. Alterations in the prolactin and thyroid stimulating hormone responses to a bolus GnRH injection after prolonged stress. Control (○--○), stress (△--△). For details, see Fig. 1.

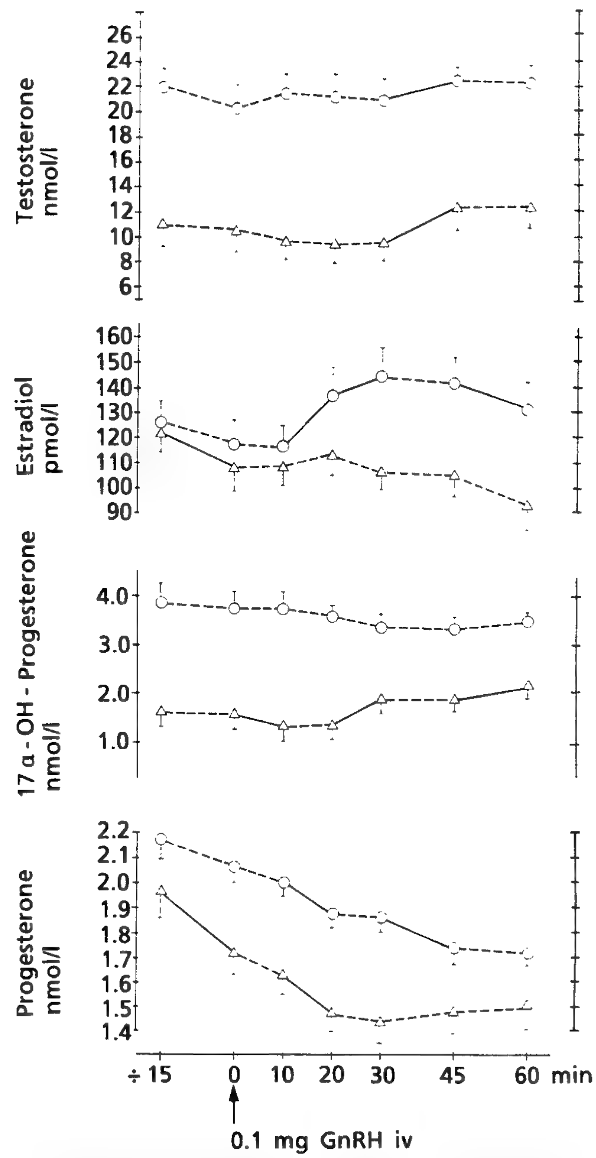

Fig. 3. Alterations in the testosterone, estradiol, 17α-OH progesterone and progesterone responses to a bolus injection of GnRH after prolonged strain. Control (○--○), stress (△--△). For details, see Fig. 1.

gesterone and dehydroepiandrosterone. For cortisol and estradiol no significant alterations were seen during the present course (Figs. 3 and 4, Table 1).

The LH response to GnRH was threefold increased at the end of the course ($F_{2.09,18.83}=9.89$, $p=0.0010$), from 2.2±0.5 to 30.5±6.4 IU/l compared to 3.3±0.7 to 15.5±3.0 in the control experiment ($F_{1.27,11.43}=18.93$, $p=0.0006$). The FSH levels increased from 9.1±0.5 to 12.4±1.2 IU/l ($F_{1.39,12.47}=18.72$, $p=0.0004$) in the control experiment and (significantly more) from 7.9±0.4 to 15.4±2.1 during the course ($F_{2.86,25.70}=5.92$, $p=0.0037$) (Fig. 1). PRL decreased from 12.7±1.8 to 8.6±1.2 µg/l ($F_{2.70,24.33}=4.59$, $p=0.0131$) after GnRH in the control experiment, whereas an increase from 4.6±0.4 to 8.6±1.3 was found during the course ($F_{4.39,39.55}=3.79$, $p=0.0088$). TSH was unaffected by GnRH in the control experiment, but increased from 0.6±0.1 to 1.0±0.1 during the course ($F_{5.51,49.59}=15.63$, $p<0.00005$).

Only minor changes were found for the different steroids after GnRH stimulation except for estradiol, which in the control experiment increased from 117±12 to a maximum level of 144±15 pmol/l after GnRH stimulation ($F_{3.80,34.17}=4.46$, $p=0.0058$), whereas a significant decrease was seen during the course ($F_{4.04,36.36}=3.65$, $p=0.0132$). During the course a slight increase in testosterone was seen 45 min after GnRH infusion ($F_{3.28,29.54}=3.53$, $p=0.0237$), and a small decrease in 17α-OH progesterone 10 min after GnRH ($F_{3.40,30.56}=2.77$, $p=0.0522$). Progesterone declined slightly during both experiments ($F_{6.00,54.00}=9.80$, $p<0.00005$), as did cortisol ($F_{3.69,33.19}=22.48$, $p<0.00005$). The androstenedione decrease was less

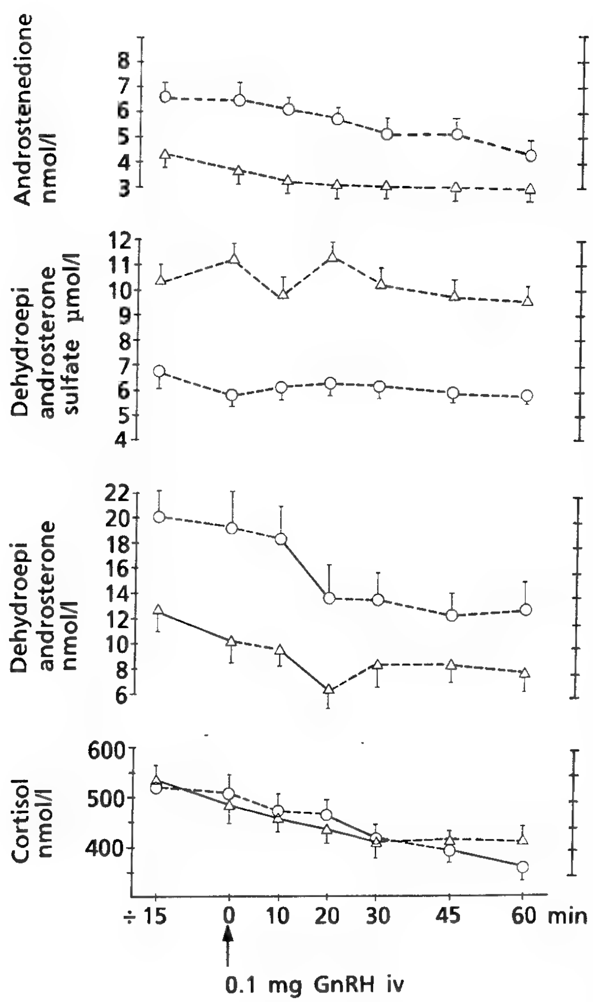

Fig. 4. The androstenedione, dehydroepiandrosterone-sulfate, dehydroepiandrosterone and cortisol responses to a bolus injection of 0.1 mg GnRH before and after prolonged stress. Control (○- -○), stress (△- -△). For details, see Fig. 1.

pronounced during the course ($F_{1.73,15.58}=11.74$, $p=0.0011$) than in the control experiment ($F_{3.82,34.40}=3.76$, $p=0.0132$). Dehydroepiandrosterone-sulfate levels were unaffected by GnRH stimulation.

During Course II a significant decrease was found for both testosterone ($t_8=20.93$, $p<0.0005$) and dihydrotestosterone ($t_8=3.97$, $p=0.004$). No significant difference was found between the levels before and after HCG stimulation (Table 2) in the control experiment, whereas a significant increase to almost control values was found after HCG stimulation during Course II for both testosterone ($t_8=10.89$, $p<0.0005$) and dihydrotestosterone ($t_8=2.46$, $p<0.039$).

Correlations: Only weak correlations were found between the different steroid hormones, as well as between the steroid hormones and the pituitary hormones, when individual samples were analyzed. By pooling the −15 and 0 min samples, and calculating correlation coefficients of the percentage alterations during the course, significant ($p<0.05$) correlations were found between LH and FSH ($r_s=0.70$), LH and PRL ($r_s=0.65$), progesterone and 17α-OH-progesterone ($r_s=0.61$), cortisol and progesterone ($r_s=0.94$) and between DHEA and DHEA-S ($r_s=0.64$).

Discussion

The increased LH/FSH response to GnRH stimulation demonstrates increased LH/FSH reserves in the pituitary gland at the end of the course and/or increased sensitivity to GnRH stimulation. These increased reserves or increased pituitary sensitivity to GnRH might be due to decreased hypothalamic GnRH secretion, which is also probably the reason for decreased plasma levels of gonadotropins. The plasma concentrations of testicular androgens during the course are therefore probably regulated through the hypothalamo-pituitary axis. This explanation is supported by an increased sensitivity of the Leydig cells to HCG stimulation, as indicated by the enhanced testosterone response during Course II. However, the fairly weak correlations between androgens and pituitary hormones weaken this hypothesis and indicate a multifactorial regulation. Weak correlations might, however, also be explained by pulsatile hormone secretion, which often makes correlation analysis of single samples difficult. Prolonged and increased exposure to plasma cortisol and catecholamines, and also decreased insulin levels, may contribute to the decreased plasma levels of testicular androgens observed (14–18) and strengthen the multifactorial regulation hypothesis. The study supports our previous findings of a decrease in all biologically active androgens, and an increase in the sulfated form (5).

PRL stimulates testicular growth, increases plasma testosterone levels and upregulates LH receptors on Leydig cells (13). The decreased PRL levels during the course may therefore contribute to the decrease in androgen levels. The decreased PRL levels after GnRH stimulation in the control experiment might be explained by the natural circadian rhythm since the experiments were performed in the morning with decreasing plasma levels of PRL. During the course the main reason for decreased PRL is physical exercise (4). Therefore the increasing plasma levels of PRL after GnRH stimulation during the course might be explained by a state of recovery since the experiments were performed with the cadets recumbent in sleeping bags. The decreased plasma TSH levels during the course are in accordance with previous investigations on the thyroid function (19). While GnRH did not affect the plasma levels of TSH in the control experiment, a significant increase was found 10 min after GnRH stimulation during the course. As for PRL this might be

Table 1. Alterations in hormones before and after five days of strenuous ranger training (Course II). The results are presented as means ± SEM. An analysis of variance for repeated measures was performed (BMDP, 4V).

	Control	Stress	Analysis of variance
LH IU/l	3.3±0.7	2.2±0.5	$F_{1,9}=11.1$, $p=0.0088$
FSH IU/l	9.2±0.7	7.8±0.5	$F_{1,9}=14.6$, $p=0.0041$
PRL μg/l	12.7±1.8	4.6±0.4	$F_{1,9}=9.0$, $p=0.0149$
TSH mIU/l	1.6±0.2	0.7±0.1	$F_{1,9}=9.6$, $p=0.0127$
Testosterone nmol/l	21.2±1.2	11.2±1.3	$F_{1,9}=7.8$, $p<0.00005$
Estradiol pmol/l	117±12	110±11	$F_{1,9}=1.1$, $p=0.3152$
17α-OH progesterone nmol/l	3.81±0.35	1.57±0.21	$F_{1,9}=35.7$, $p=0.0002$
Progesterone nmol/l	2.12±0.12	1.54±0.08	$F_{1,9}=6.3$, $p=0.03331$
Androstenedione nmol/l	6.7±0.5	4.3±0.3	$F_{1,9}=34.0$, $p=0.0002$
Dehydroepiandrosterone (DHEA)	19.6±2.2	11.2±1.2	$F_{1,9}=9.0$, $p=0.0148$
DHEA—Sulfate μmol/l	6.7±0.8	10.7±1.1	$F_{1,9}=16.0$, $p=0.0027$
Cortisol nmol/l	505±25	489±30	$F_{1,9}=0.1$, $p=0.6908$

Table 2. Dihydrotestosterone and testosterone levels in plasma before (08.00) and after (11.00) intramuscular injection of 3000 IU of human chorion gonadotropin (HCG) in a control experiment and after five days of continuous military activities. The results are presented as means ± SEM.

	Control		Stress	
	Before HCG	3-h after HCG	Before HCG	3-h after HCG
Dihydrotestosterone nmol/l	2.6±0.5	2.4±0.6	0.4±0.2	1.2±0.2
Testosterone nmol/l	26.0±1.1	24.6±1.3	2.4±0.2	17.3±1.5

explained by the physical rest the subjects got during the experiment.

Testosterone and dihydrotestosterone originate mainly from the testes, whereas the other androgens, such as DHEA, 17α-OH progesterone and androstenedione, are produced by the testes and by the adrenals (20). Normally only 10–25% of circulating DHEA, 25–30% of androstenedione and 10% of 17α-OH progesterone derive from direct testicular secretion (20–22). A total stop in all testosterone production would not be enough to explain the decrease in the androgen secretion during the course. Therefore both testicular and adrenal androgens must decrease.

Cortisol, androstenedione, DHEA-S, 17α-OH progesterone and progesterone were all unaffected by GnRH stimulation. DHEA showed a 30 to 40% decrease from 10 to 20 min after the GnRH stimulation, and testosterone increased 10 to 20% from 30 to 45 min after GnRH stimulation. This delayed response might indicate that GnRH via LH, stimulates the enzymes converting DHEA to testosterone.

The divergence observed between cortisol and adrenal androgens supports the hypothesis of separate regulators for glucocorticoid and adrenal androgen secretion during prolonged physical stress. Other situations with a divergence between cortisol and the adrenal androgens are after corticoid treatment when cortisol normalizes much more rapidly than the androgen secretion, the increase in adrenal androgen secretion before puberty and its corresponding decrease in old age, without concomitant alterations in cortisol secretion. During severe illness, such as in patients with burns and trauma, there is also a decrease in adrenal androgens and an increase in glucocorticoids. However, up till now the existence and nature of a specific factor regulating the adrenal androgens remains hypothetical, although Odell and Parker (23) reported the isolation of a pituitary polypeptide different from ACTH that selectively stimulated adrenal androgen secretion (22).

The divergence between the biologically active adrenal androgens and the sulfated form (DHEA-S) may be explained by different adrenal secretion rates, particularly since 80% of DHEA and 90–98% of DHEA-S derive from direct adrenal secretion (24). It may also be explained by an inhibition of the desulfation of DHEA-S and a stimulation of the sulfokinase in peripheral tissues acting upon circulating DHEA, or also by all three mechanisms. The positive correlation between DHEA and DHEA-S shows that the subjects with the smallest decrease in DHEA had the highest increase in DHEA-S.

Only about 10% of plasma estradiol derives from direct secretion from the Leydig cells. About 80–90% derives from peripheral conversion of androstenedione and testosterone to estradiol. Most of it takes place in the fat cells (25). In addition, the Sertoli cells may convert androgens to estradiol. The inreased plasma level of estradiol after GnRH stimulation is probably mediated via the increased LH/FSH secretion and may take place in the Sertoli cells. During the course the estradiol response to the increased LH/FSH response is completely inhibited, indicating decreased sensitivity of aromatase in Sertoli cells or in fat cells for LH/FSH stimulation (26).

The decreased secretion of dihydrotestosterone and testosterone during the course is probably due to reduced LH/FSH stimulated testosterone secretion rather than a primary testicular defect, since the testosterone levels were normalized 3 h after HCG stimulation.

The lack of increase in the plasma levels of testosterone after HCG stimulation in the control experiment might be due to the circadian rhythm of this hormone since a decrease should have been expected without HCG stimulation (13, 27, 28).

In conclusion, both adrenal and testicular androgens decrease during prolonged physical stress, sleep and energy deficiency. The decrease in testicular androgens is mainly regulated through the hypothalamo-pituitary axis.

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PAPER VII

Circadian rhythm of hormones is extinguished during prolonged physical stress, sleep and energy deficiency in young men

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The circadian rhythm of hormones (N = 10) and mental performance (N = 18) was investigated in male cadets during a 5-day military training course with continuous heavy physical activities corresponding to 35% of the maximal oxygen uptake, with almost total lack of food and sleep. The 24-h means for androstenedione, dihydroepiandrosterone (DHEA), 17α-hydroxyprogesterone, testosterone and thyroid-stimulating hormone decreased strongly during the course, and the circadian rhythm was extinguished below the minimum levels measured during the control experiment. The 24-h means for cortisol, dihydroepiandrosterone sulfate (DHEA-S) and progesterone increased during the course, and the circadian rhythm was abolished above the maximum levels of the control experiment. A gradual increase was found in thyroxine, free thryoxine and triiodothyronine during the first 12 h of activities, followed by a constant decrease for the rest of the course. Mental performance decreased during the course and the amplitude of its circadian rhythm increased from ±10% to ±30% of the 24-h mean. The circadian rhythms investigated were almost normalized after 4–5 days of rest. However, the nocturnal rise for cortisol, androstenedione and DHEA appeared earlier, and the plasma levels of thyroid hormones, estradiol and DHEA-S were lower during the recovery experiment than in the control experiment. The responses to stress of the circadian rhythm for mental performance and steroid hormones during the course indicate a differential regulation.

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Homeostasis is one of the major principles in the organization of biological systems and provides constancy of a variable function in its long-term mean (1–3). In addition, almost all cells and biological systems have oscillations around this mean in rhythms of various frequencies from one cycle per millisecond to one cycle per several years. These rhythms can be observed in single cells, in networks of tissues and organs as well as in whole organisms and even in populations.

Basically it is necessary to distinguish between two types of rhythms: the exogenous rhythm, which only reflects the biological response to the environment input; and the endogenous rhythm, which is inborn in the organism, persists in a milieu deprived of all external influences and is self-sustained.

In humans, a broad spectrum of biological rhythms has been observed: an ultradian rhythm with a frequency of less than 24 h, an infradian rhythm with a frequency longer than 24 h and finally the circadian rhythm with approximately a 24-h cycle. Although most cells of the body exhibit their own rhythm, structures in the central nervous system (CNS) are thought to synchronize the different rhythms.

Free-run studies, where the subjects are deprived of all external inputs, and also observations of subjects submitted to progressive shortening of artificial time cues, have shown that parameters such as alertness can become independent of the sleep/wake cycle and body temperature (4). However, dissociation in the amplitude of the circadian rhythm is not described.

The circadian rhythm of human growth hormone (hGH) is connected to slow-wave sleep, whereas the adrenal and testicular steroids have their own endogenous circadian rhythms independent of sleep (5–12). Information on the circadian rhythm of catecholamines is contradictory (13–19) and the circadian variations in the thyroid hormones are small (20–23).

Previously we have studied the effect of prolonged hard physical exercise combined with sleep and energy deprivation on the circadian rhythm of mental performance and mood in healthy young men during a military training course (24). The oscillation in performance and mood increased from 10–15% normally to 20–40% during the course, with a crest in the afternoon and a trough in the early morning hours. The decrement in performance during the course therefore was much more pronounced during night-time than during the daytime.

Considerable alterations also have been demonstrated for several hormones and metabolites during similar courses (25–29). There is an increase in glucocorticoids, mineralocorticoids and catecholamines and a decrease in the testicular hormones. The thyroid hormones show a biphasic pattern with an initial

increase during the first day of activities, followed by a gradual decrease for the rest of the course.

There is, however, no information on the influence of extreme stress on the endocrine circadian rhythm. During the present course we wanted to study how short- and long-lasting stress, including physical exercise and sleep and energy deficiency, influences the circadian rhythm of hormones. The subsequent recovery also studied.

Materials and methods

Subjects and the course

The subjects of this investigation were cadets of the Norwegian Military Academy participating in a military training course as part of their school program. The course started on a Monday at 08.00 h and ended on the following Friday at 18.00 h. The cadets were between 22 and 26 years of age. The cadets are selected to the Academy on the background of psychological tests, physical performance and previous results. They are in excellent physical and mental condition.

The cadets had continuous physical exercise (infantry activities) around the clock, corresponding to about 35% of the maximal oxygen uptake ($\dot{V}_{O_2,max}$) and a calorie consumption of about $40\,000\,kJ \cdot 24\,h^{-1} \cdot cadet^{-1}$. The energy intake was about $5000\,kJ \cdot 24\,h^{-1} \cdot cadet^{-1}$, of which 70% was carbohydrates (bread). In addition, two cadets shared one cooked chicken on day 4 during the course. On day 1 before the start of the course, the subjects had a normal breakfast after blood sampling. The intake of water was free during the course, but in spite of this the subjects complained of thirstiness. The subjects had a 4–6-kg reduction of body weight during the course, of which 3–4 kg was body fat. No organized sleep was allowed, but the cadets caught small periods (minutes) of sleep between activities, estimated to a total of 1–3 h during the whole course.

The course was run in a forest area in the eastern part of Norway (60.8° North, 11.5° East) at about 500 m altitude in June. The weather was mostly good and sunny, warm by day (20–25°C) and cool at night (4–8°C). On Thursday it was cloudy with shorter periods of rain. At this latitude light intensity may reach 100 000 lx in the middle of a sunny day, whereas on cloudy days light intensity is about 10 000 lx. Over a 5-h period at night the light intensity is below 200 lx.

Baseline and recovery experiments were performed while the subjects had ordinary school activities in the week before and after the course. In the classroom the light intensity is more than 1000 lx. The subjects were allowed normal sleep at night. Sleeping time was 23.00–07.00 h in the baseline test, whereas during the recovery test bed-time was advanced by 1–2 h. In addition, the cadets had small naps during the day in the days after the course.

The circadian rhythms were studied once in the week prior to the course (baseline), on the first day of the course (day 1–2), on the last day of the course (day 4–5) and then 4–5 days after the end of the course (recovery).

Eighteen cadets were tested with two mental performance tests lasting for about 5 min each, succeeded by 10–15 min of rest before blood sampling. The same procedure was followed each time. Mental performance testing and blood sampling were performed seven times during 24 h, from 08.00 h and at approximately 4-h intervals. Owing to military activities there were some slight deviations in the time schedule during the course. The cadets were given only a few small meals during the course. The meals were consumed just after blood sampling in order to reduce the possible influence of eating on the blood levels of hormones and metabolites.

Mental performance

Mental performance was measured with two standard mental performance tests: the code test and the logical reasoning test. The cadets were tested five times before the course in order to reach the plateau of the learning curve. The results are given as per cent of the first control test results (control at 08.00 h). The code test is a digit-symbol test and originates from the "Otis Army Beta Battery" (31). The test is assumed to test visual acuity, motor coordination and speed of association. The logical reasoning test is assumed to test "higher mental processes" (32). The test comprises sentences claiming to describe the order of two letters (A and B), and the subjects have to decide whether the description is true or not.

Blood sampling

The blood was drawn from the antecubital vein of 10 (selected at random) of the 18 cadets participating in the course, with the cadets sitting. Blood for plasma was collected into ice-chilled vacuum tubes containing 143 USP units of sodium heparin. The tubes were kept on ice until centrifuged in a refrigerated centrifuge within 30 min at 3000 *g* for 15 min. Blood for serum was collected in vacuum tubes, allowed to clot at room temperature and then centrifuged after about 30 min. Plasma and serum were frozen on dry ice immediately after centrifugation and kept frozen at −80°C until analyzed.

Chemical analysis

For the radioimmunoassays all samples were analyzed in the same assay so that the bias due to interassay variations was eliminated. For the catecholamines, all samples from one to three cadets were analyzed simultaneously, which also reduced the bias due to interassay variations. The intra-assay percentage coefficients of variation (cv) are given in parentheses.

The three catecholamines dopamine (10.4), noradrenaline (6.2) and adrenaline (7.8) were analyzed in heparinized plasma with a radioenzymatic method (30). Cortisol (6.6) was analyzed with a GammaCout RIA kit from Clinical Assays. Progesterone (7.5), estradiol (6.9), testosterone (6.1), dihydroepiandrosterone (DHEA, 7.7), and 17α-hydroxy-progesterone (6.1) were analyzed with coat-a-count kits from Diagnostic Products Corporation. Dihydroepiandrosterone sulfate (DHEA-S, 3.1) and androstenedione (5.9) were analyzed with solid-phase RIA kits from Diagnostic Systems Laboriatores Inc. Thyroxine (T_4, 3.1), free thyroxine (FT_4, 3.1), triiodothyronine (T_3, 2.1) and free triiodothyronine (FT_3, 8.3) were analyzed with double-antibody RIA kits from Diagnostic Product Corporation. Thyroid-stimulating hormone (TSH, 6.9) was analyzed with a GammaDab RIA kit from Clinical Assays, and human growth hormone (7.9) with a double-antibody kit from Immunonuclear Corp. Glucose (3.2) was determined with the hexokinase method, using a kit from Boehringer, Mannheim, Germany.

Statistics

All the cadets were treated as one group. The results are presented as means ± SEM. The presence of a circadian rhythm, the overall effects of stress and effects of stress on the circadian rhythm were tested with an analysis of variance for repeated measures (BMDP, 4V). Significant differences ($p < 0.01$) between time points were calculated according to Student's *t*-test and are indicated in the figures by thick lines, whereas no significance is indicated by the dotted lines. Cosine analyses were not performed owing to infrequent sampling.

Results

Cortisol (nmol/l) (Fig. 1)

In the control experiment, serum cortisol decreased during the day to a nadir of 72 ± 14 at 24.00 h followed by an increase to 478 ± 43 at 08.00 h. The 24-h mean was 283 ± 24 ($F_{4.07,36.67} = 46.56$; $p < 0.00005$). At 08.00 h just before the start of the course the levels were

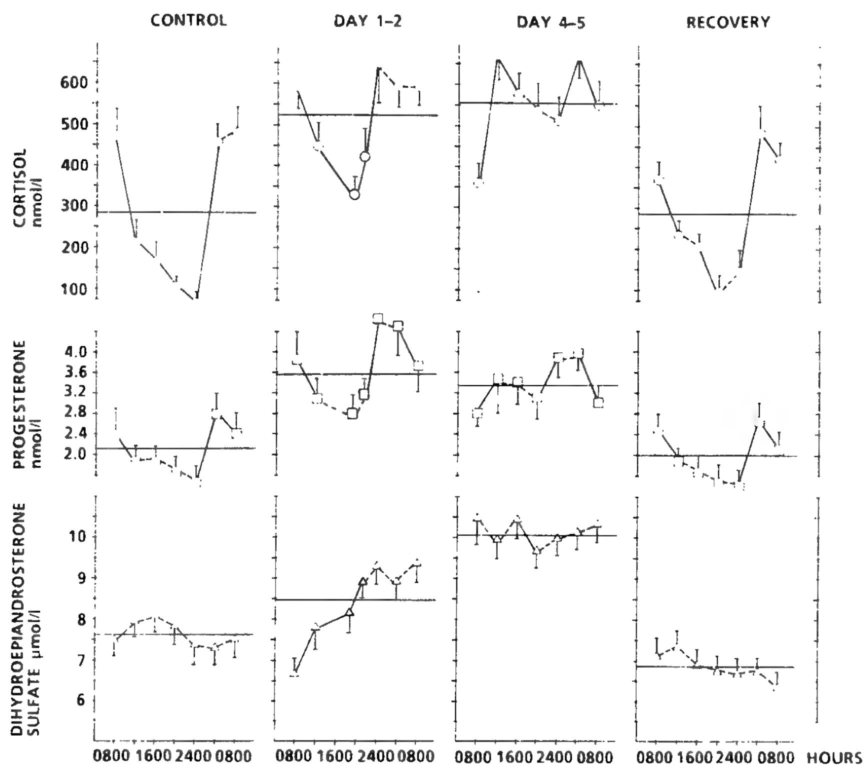

Fig. 1. The circadian rhythm for cortisol, progesterone and dihydroepiandrosterone sulfate during a control experiment (left column) with normal school activities, during the first 24 h of continuous activity (mid-left column) and from 72 to 96 h of activities (mid-right column) during a military training course with continuous physical activities almost without sleep and with limited amounts of food. The recovery experiment (right column) was performed 4–5 days after the course, while the cadets had normal school activities. The blood samples were collected at 4-h intervals. The results are expressed as means ± SEM. The time-to-time variations that were statistically significant at $p < 0.01$ are shown with thick lines and those that were not significant by dotted lines. Horizontal lines indicate 24-h means.

significantly higher than in the control experiment, probably because of anxious anticipation before a strenuous course. From 08.00 h to 16.00 h on day 1, the expected decrease was seen. From 24.00 h and through the rest of the course the levels were increased to about 130–140% of normal morning levels ($F_{3.00,27.00} = 44.46$; $p < 0.00005$). The 24-h mean of day 1–2 was 519 ± 29. During the last 24 h the circadian rhythm was almost extinguished, and the 24-h mean was 556 ± 30. In the recovery experiment the 24-h mean had normalized to 287 ± 25, whereas the circadian rhythm of plasma cortisol was still different from the control experiment ($F_{5.43,45.88} = 4.46$; $p = 0.0016$).

Progesterone (nmol/l) (Fig. 1)

Progesterone also showed a circadian rhythm, with a decrease during day time to a minimum level of 1.56 ± 0.25 at 24.00 h followed by an almost twofold increase at night to 2.80 ± 0.29 at 04.00 h ($F_{4.82,38.57} = 7.05$; $p = 0.0001$). The 24-h mean was 2.16 ± 0.12. During the first day of activities a decrease was seen until 16.00 h, followed by an increase to a maximum level already at 24.00 h instead of at 04.00 h in the control experiment ($F_{6.00,48.00} = 8.06$; $p < 0.00005$). All levels were higher during the course than in the control and recovery experiments ($F_{2.68,21.44} = 8.06$; $p < 0.00005$), and the 24-h mean was 3.53 ± 0.16 on day 1–2 and 3.35 ± 0.14 on day 4–5. During the last day of the course the circadian rhythm of progesterone was almost extinguished. In the recovery experiment the circadian rhythm of progesterone was normalized and the 24-h mean was 2.04 ± 0.11.

Dihydroepiandrosterone sulfate (DHEA-S) (mmol/l) (Fig. 1)

Dihydroepiandrosterone sulfate showed only a very moderate circadian rhythm with a maximum level at 16.00 h and a minimum level at 04.00 h ($F_{6,54} = 3.76$; $p < 0.0034$). The 24-h mean was 7.70 ± 0.20. During the first day of the course a gradual increase was seen ($F_{4.95,44.51} = 12.59$; $p < 0.00005$). The 24-h mean was 8.11 ± 0.24. On the last day of the course there was no circadian rhythm, with a 24-h mean of 9.63 ± 0.28. No significant alterations were seen during the recovery experiment. The 24-h mean was 6.57 ± 0.27.

Androstenedione (nmol/l) (Fig. 2)

Adrostenedione showed a circadian rhythm with a minimum level of 4.5 ± 0.5 at 24.00 h and a twofold increase to 10.8 ± 0.9 at 08.00 h ($F_{6,42} = 15.62$; $p < 0.00005$). The 24-h mean was 7.9 ± 0.3. During day 1 a gradual decrease was found until 16.00 h ($F_{5.31,37.19} = 7.5$; $p < 0.00005$). The nocturnal increase was, however, almost absent. The 24-h mean

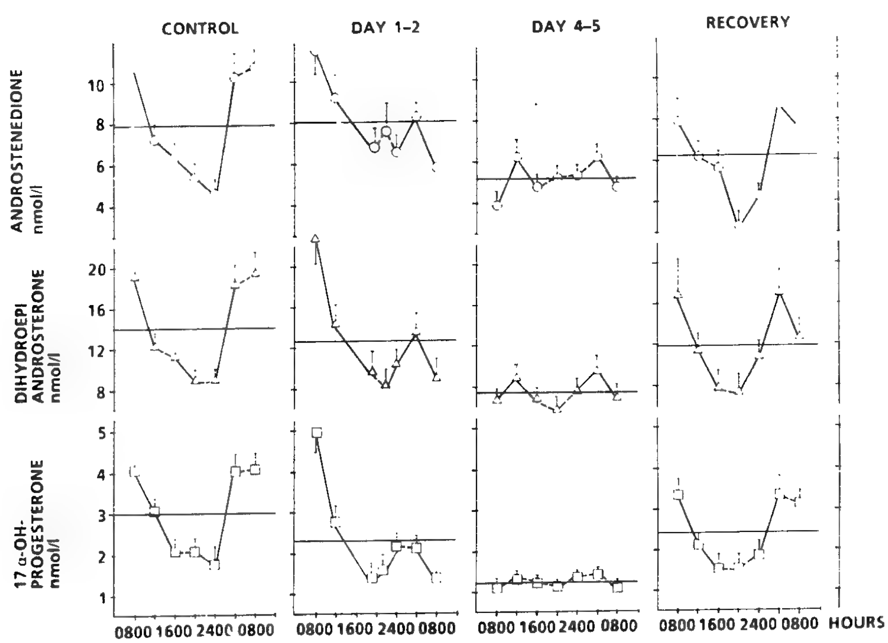

Fig. 2. The circadian rhythm for androstenedione, dihydroepiandrosterone and 17α-hydroxyprogesterone during a control experiment (left column), during the first 24 h of continuous activities (mid-left), from 72 to 96 h of activities (mid-right) and during recovery (right column). For details, see Fig. 1

was 8.0 ± 0.4. During the last day of the course the circadian rhythm was almost extinguished at the lowest levels found during the control experiment ($F_{4.89,34.21} = 2.75$; $p = 0.0353$). The 24-h mean was 5.1 ± 0.3. In the recovery experiment the circadian rhythm was still not re-established ($F_{5.34,37.35} = 23.91$; $p < 0.00005$) and the 24-h mean was 6.2 ± 0.4.

Dihydroepiandrosterone (DHEA) (nmol/l) (Fig. 2)

In the control experiment the plasma levels of DHEA decreased gradually during daytime to a minimum level of 8.8 ± 1.0 at 20.00 h and a maximum level of 19.2 ± 3.8 at 08.00 h ($F_{2.47,17.30} = 6.52$; $p < 0.0054$) and the 24-h mean was 14.0 ± 1.1. During the first day of activities a gradual decrease was found from 23.6 ± 2.2 at 08.00 h to 8.5 ± 1.6 at 20.00 h ($F_{5.1,35.97} = 12.14$; $p < 0.00005$). The nocturnal increase was, however, strongly reduced ($F_{2.83,19.84} = 11.22$; $p = 0.0002$). The 24-h mean was 12.6 ± 0.9. On the last day of the course the circadian rhythm was almost abolished ($F_{6.00,42.00} = 2.54$; $p = 0.0346$) and the 24-h mean was 3.7 ± 0.3. In the recovery experiment the circadian rhythm was re-established and the 24-h mean was 11.8 ± 0.8.

17α-Hydroxyprogesterone (nmol/l) (Fig. 2)

17α-Hydroxyprogesterone in serum decreased during daytime in the control experiment to a minimum level of 1.7 ± 0.3 at 24.00 h followed by a nocturnal increase with a maximum level of 4.1 ± 0.4 at 08.00 h ($F_{3.24,29.12} = 18.76$; $p < 0.00005$). The 24-h mean was 3.0 ± 0.3. During day 1–2 the levels decreased from 4.9 ± 0.5 at 08.00 h to a minimum level of 1.4 ± 0.2 at 16.00 h. The nocturnal rise was strongly reduced and the 24-h mean was 2.3 ± 0.2. During the last day of the course the circadian rhythm was abolished and the 24-h mean was 1.2 ± 0.2. In the recovery experiment the circadian rhythm was still not re-established ($F_{5.06,45.53} = 16.00$; $p < 0.00005$). The levels were significantly lower than in the control experiment ($F_{1,9} = 15.49$; $p = 0.0034$), with a 24-h mean of 2.4 ± 0.3.

Testosterone (nmol/l) (Fig. 3)

In the control experiment the serum level of testosterone decreased to a minimum of 15.0 ± 2.1 at 24.00 h, followed by a nocturnal increase to a maximum level of 21.6 ± 1.6 at 08.00 h ($F_{3.81,34.29} = 11.69$; $p < 0.00005$). The 24-h mean was 18.8 ± 1.5. During the first day of the course testosterone decreased gradually to 5.6 ± 0.8 at 08.00 h on Day 2 ($F_{3.31,29.86} = 20.45$; $p < 0.00005$). There was no increase during night-time. On the last day of the course the 24-h mean was 5.9 ± 0.5 with no circadian rhythm. In the recovery experiment the circadian rhythm was still not re-established in that the nocturnal increase started earlier than in the control experiment ($F_{3.85,34.63} = 2.90$; $p = 0.0378$). The 24-h mean was 17.4 ± 1.8.

Estradiol (nmol/l) (Fig. 3)

Estradiol levels in serum did not show any significant circadian rhythm and no significant alterations were found in the plasma levels of estradiol during the course. However, in the recovery experiment significantly lower levels were found than during the course or in the control experiment ($F_{1,8} = 5.40$; $p = 0.0486$). The 24-h mean was 0.145 ± 0.01 in the control experiment, 0.159 ± 0.01 during day 1–2, 0.151 ± 0.01 during the last day and 0.105 ± 0.01 during recovery.

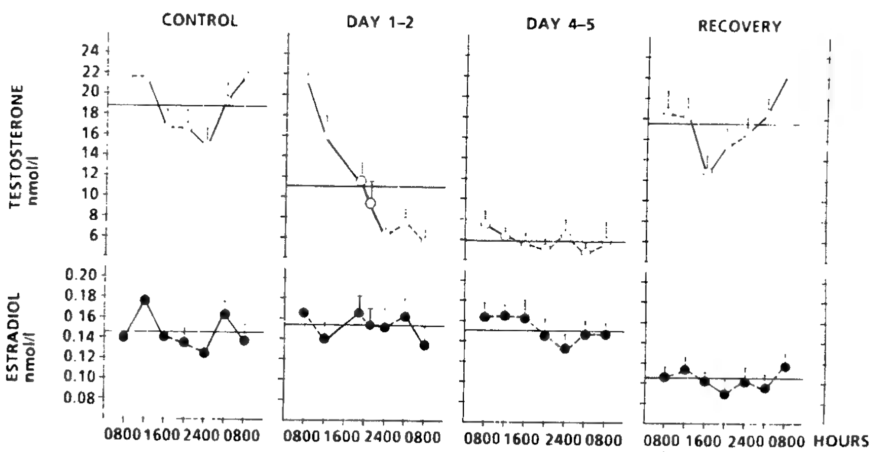

Fig. 3. Alterations in the circadian rhythm for testosterone and estradiol in a control experiment, during short and prolonged continuous stress and during recovery. For details, see Fig. 1.

Noradrenaline (nmol/l) (Fig. 4)

The concentration of noradrenaline in plasma did not show any significant circadian rhythm in this study. A gradual increase was found during the first day of activities from 2.2 ± 0.2 at 08.00 h before the start of the course to 6.9 ± 1.5 at 04.00 h ($F_{1.90,17.09} = 6.88$; $p < 0.007$). During the last day the variations in noradrenaline were probably due mainly to varying physical activities. The 24-h mean increased from 2.0 ± 0.2 in the control experiment to 5.1 ± 0.3 during the first day, 7.7 ± 0.5 on day 4–5 and 2.5± 0.2 during recovery ($F_{1.87,16.81} = 10.55$; $p = 0.0013$).

Adrenaline (nmol/l) (Fig. 4)

No circadian rhythm was found for adrenaline during the course. The 24-h mean was 0.88 ± 0.08 in the control experiment. During day 1–2 the levels increased gradually from 1.06 ± 0.35 at 08.00 h to 2.78 ± 1.18 at 04.00 h ($F_{6,54} = 2.62$; $p = 0.0265$) and the 24-h mean was 2.05 ± 0.33. Further, the 24-h mean was 1.36 ± 0.21 on day 4–5 and 0.66 ± 0.07 during the recovery day.

Dopamine (nmol/l) (Fig. 4)

Dopamine did not show any significant circadian rhythm. The 24-h mean increased from 0.52 ± 0.06 in the control experiment to 1.05 ± 0.15 on day 1–2, and decreased to 0.62 ± 0.05 on day 4–5 and further to 0.56 ± 0.04 during recovery.

Glucose (mmol/l) (Fig. 5)

The 24-h glucose means were 5.0 ± 0.1 in the control experiment, 4.9 ± 0.1 during day 1–2, 5.1 ± 0.1 during day 4–5 and 5.0 ± 0.1 during recovery. In spite of the fact that the cadets were instructed not to eat before blood sampling, the variations in blood glucose levels in the day 1 samples taken at 24.00 h are probably due to preceding food intake.

Human growth hormone (μg/l) (Fig. 5)

Human growth hormone in blood increased during the course from a 24-h mean of 2.0 ± 0.3 in the control experiment to 3.4 ± 0.3 during day 1–2 and to 5.0 ± 0.3 during day 4–5 ($F_{3.00,27.00} = 18.61$; $p < 0.00005$). The 24-h mean of the recovery day was 1.9 ± 0.2. No apparent circadian variations were seen during the control or recovery experiment. The gradual increase seen during the first day of activities and the variations in hGH during the last day of the course were probably due to preceding physical activities. The sample taken at 08.00 h in the recovery experiment contains significantly more hGH than that taken at the other time points.

Thyroid-stimulating hormone (mIU/l) (Fig. 5)

In the control experiment TSH in plasma increased from a minimum level of 4.7 ± 0.4 at 12.00 h to a maximum level of 8.0 ± 1.0 at 24.00 h ($F_{3.21,19.28} = 9.67$; $p = 0.0003$). During the first day of activities a gradual decrease was found from 6.1 ± 0.9 at 08.00 h just before the start of the course to 3.5 ± 0.4 after 24 h of activities ($F_{2.93,17.57} = 10.32$; $p = 0.0004$). During the course there was no nocturnal rise in the plasma levels of TSH. During the recovery experiment the circadian rhythm was re-established, but the levels were still

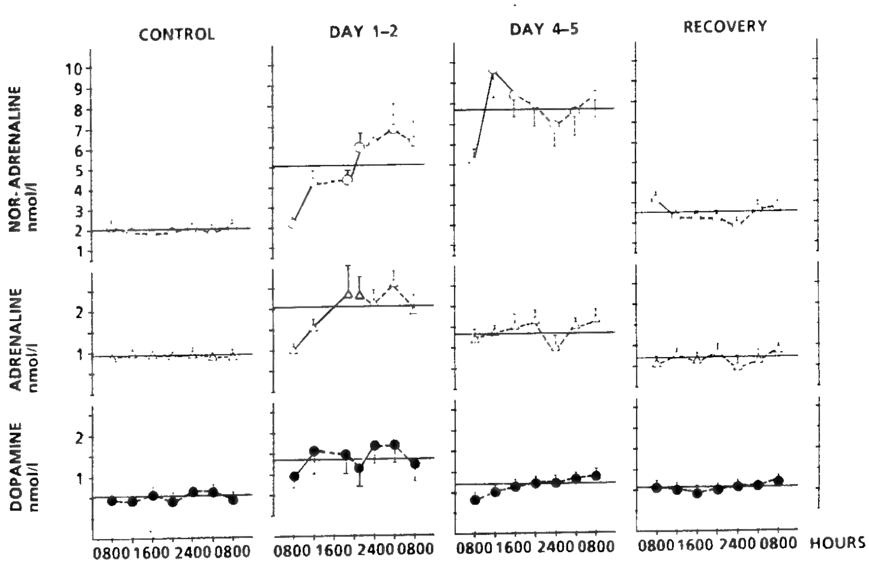

Fig. 4. Changes in the circadian rhythm for plasma noradrenaline, adrenaline and dopamine in a control experiment, during stress and during recovery. For details, see Fig. 1.

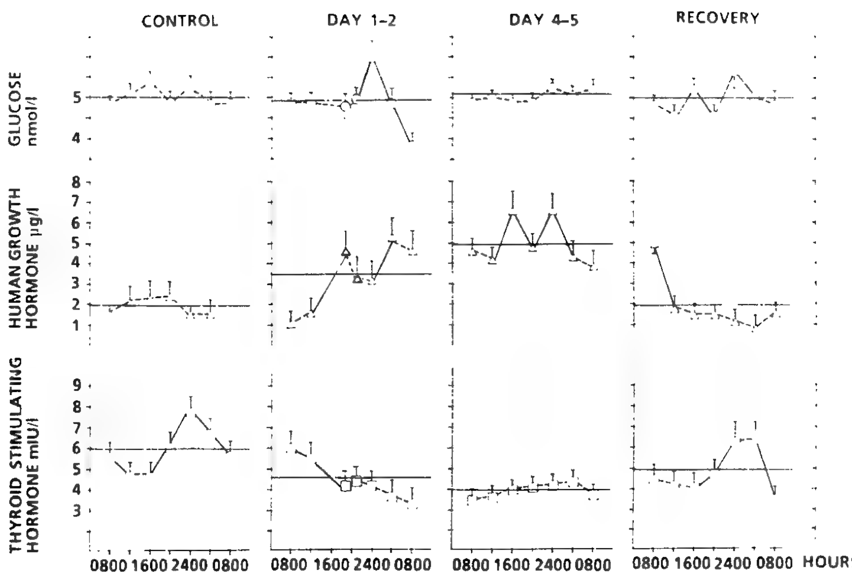

Fig. 5. Alterations in the circadian rhythm of plasma thyroid-stimulating hormone, human growth hormone and glucose during short-term and long-term stress. For details, see Fig. 1.

lower than control levels ($F_{1,6} = 23.29$; $p = 0.0029$). During the last day of the course the levels were significantly lower than for the rest of the experiment ($F_{1,6} = 98.74$; $p = 0.0001$). The 24-h mean was 6.0 ± 0.2 in the control experiment, 4.6 ± 0.2 on day 1–2, 4.0 ± 0.2 on day 4–5 and 4.9 ± 0.2 in the recovery experiment.

Thyroxine (nmol/l) (Fig. 6)

Thyroxine in serum did not show any circadian rhythm during the control or the recovery experiment. On day 1 at 08.00 h the T_4 level was significantly lower than in the control experiment ($F_{1,8} = 17.65$; $p < 0.0030$). During the first day of activities plasma T_4 increased from 83.2 ± 5.0 at 08.00 h to a maximum level of 106 ± 3.5 at 24.00 h ($F_{15.03,40.27} = 12.76$; $p < 0.00005$). During the last day of the course all levels were significantly lower than in the control and recovery experiment ($F_{1,8} = 29.16$; $p = 0.0006$). The 24-h mean was 94.3 ± 1.8 in the control experiment, 97.6 ± 1.8 on day 1–2, 77.3 ± 2.1 on day 4–5 and 95.4 ± 1.9 in the recovery experiment.

Free thyroxine (pmol/l) (Fig. 6)

In the control experiment and during recovery FT_4 did not show any significant circadian variations. During the first day of activities FT_4 increased from 16.9 ± 1.1 at 08.00 h to a maximum level of 19.9 ± 1.8 at 24.00 h ($F_{4.58,32.07} = 6.16$; $p = 0.0006$). During the last day of the course all levels were significantly lower than during the other days ($F_{1,7} = 14.61$; $p = 0.0065$). The 24-h mean was 17.5 ± 0.6 in the control experiment, 18.2 ± 0.5 during day 1–2, 14.6 ± 0.7 during day 4–5 and 18.0 ± 0.7 in the recovery experiment.

Triiodothyronine (nmol/l) (Fig. 6)

No circadian variation was seen for T_3. The 24-h mean was 1.97 ± 0.04 in the control experiment, 2.06 ± 0.04 during day 1–2 and 1.38 ± 0.02 during day 4–5 ($F_{2.40,16.83} = 36.32$; $p < 0.00005$). During recovery the 24-h mean was 1.78 ± 0.02 and all levels were still lower than the control levels ($F_{1,7} = 6.89$; $p = 0.0341$). On day 1 just before the start of the course, the levels were significantly lower than in the control experiment ($F_{1,7} = 18.05$; $p = 0.0038$). An increase was seen during day 1 from 1.77 ± 0.11 at 08.00 h to 2.14 ± 0.07 at 20.00 h ($F_{6,42} = 2.62$; $p = 0.0302$). On the last day of the course all levels were significantly lower than during the other days ($F_{1,7} = 42.05$; $p = 0.0003$).

Free triiodothyronine (pmol/l) (Fig. 6)

No significant circadian variation in FT_3 was seen during the investigation. A gradual decrease was observed during the first 24 h of activities ($F_{4.79,43.10} = 5.12$; $p = 0.001$). On the last day of the course the levels were significantly lower than during the days ($F_{1,7} = 42.05$; $p = 0.0003$). The 24-h mean was 3.64 ± 0.12 in the control experiment, 3.88 ± 0.08 during day 1–2, 2.39 ± 0.08 during day 4–5 and 3.24 ± 0.09 in the recovery experiment ($F_{1.79,16.13} = 13.17$; $p = 0.00005$).

Mental performance (Fig. 7)

Mental performance decreased dramatically during the

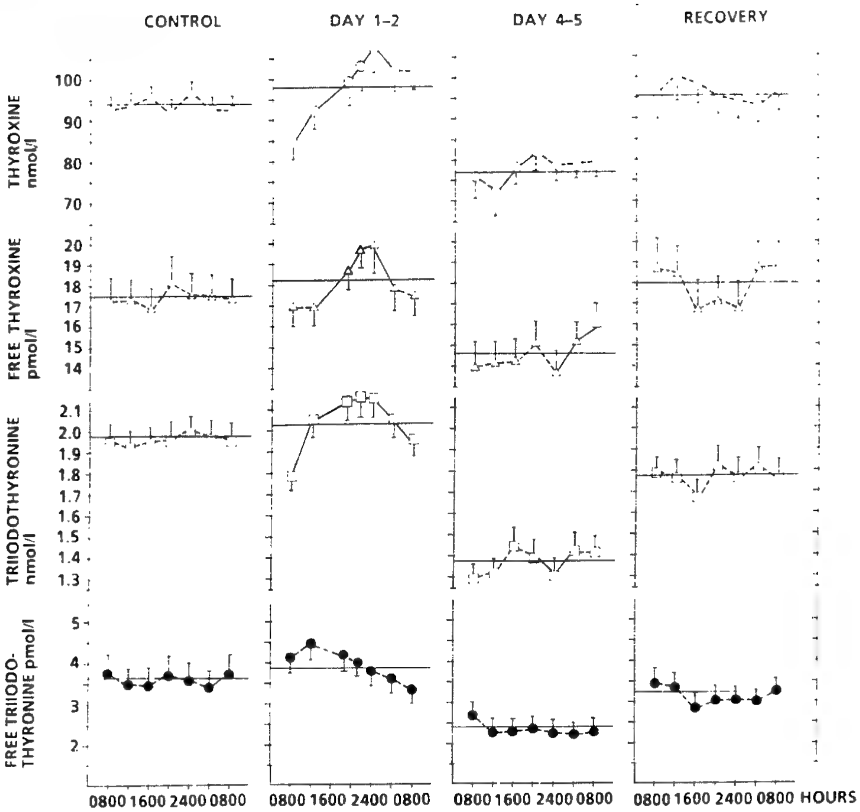

Fig. 6. The influence of short and prolonged stress on the circadian rhythm for thyroxine, free thyroxine, triiodothyronine and free triiodothyronine. For details, see Fig. 1.

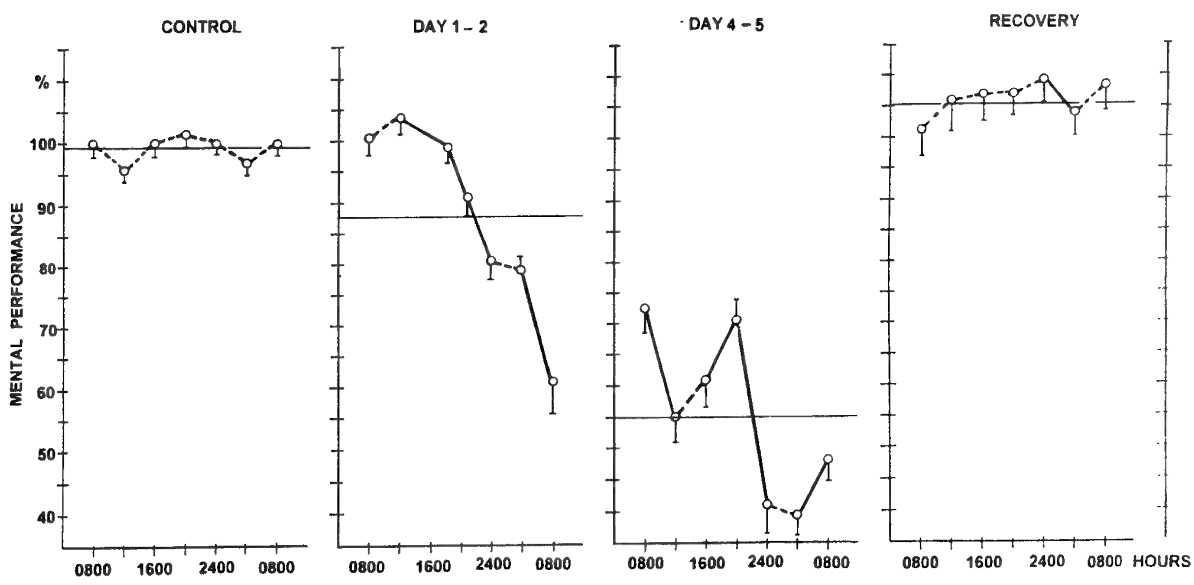

Fig. 7. The circadian rhythm for mental performance expressed as the mean of two mental performance tests: the code test and the logical reasoning test. The results are presented as per cent of the first test results (control at 08.00 h) ± SEM. For details, see Fig. 1.

course ($F_{2.17,73.62} = 63.55$; $p < 0.00005$). The circadian rhythm showed low levels in the early morning hours ($F_{4.56,155.16} = 38.80$; $p < 0.00005$) and its amplitude was strongly increased during the course ($F_{12.20,414.74}$; $p < 0.00005$). The decrease in mental performance was therefore much stronger during night-time than during daytime. Mental performance and its circadian rhythm was re-established 4–5 days after the course.

Discussion

The present paper confirms previous publications showing that all steroid hormones exhibit a circadian rhythm, with the lowest levels during daytime and maximal levels during night-time (for a review, see Krieger (11) and Touitou and Haus (33)). The results show that the nocturnal increase in the steroid hormones is strongly reduced already during the first night of activities and abolished after 4–5 days of continuous hard physical stress and sleep and energy deficiency. Estradiol is the only steroid hormone investigated that did not show any circadian rhythm.

Bright light is one of the strongest "zeitgebers" known. There is no reason to believe that different light conditions in the four experiments could explain the present results. The hormone most sensitive to environmental light, melatonin, is completely inhibited by 2500 lx or more, and is not influenced by light weaker than 500 lx (33). The light intensity is lower than 200 lx for at least 5 h at night-time at this latitude, even in summertime, and is more than 10 000 lx during a cloudy day. Because the cadets in the control and recovery experiments only passed some hours each day in classroom teaching (>1000 lx), the differences in light intensity between the four experiments was not important for the present results.

The dissociation between the circadian rhythm for mental performance and plasma levels of steroid hormones during stress supports the hypothesis that more than one cerebral clock normally regulates the circadian rhythms and that these clocks are different for mental performance and the steroid hormones.

The 24-h means for cortisol, progesterone and DHEA-S, which originate almost exclusively from the adrenals, increase during the course and the circadian rhythms are extinguished, but at levels above the maximum level of the normal circadian rhythm. The increases mainly take place during daytime, whereas the normal nocturnal increases are strongly reduced or absent. Physical exercise and energy deficiency are known to stimulate cortisol secretion and might be the cause of the increased levels during the course, compensating for the abolished circadian rhythm.

For plasma testosterone, 97% of which originates from the testes (34), the circadian rhythm is also extinguished, but at a lower level than the minimum level of the normal circadian rhythm. This indicates an inhibition of both night-time and basal androgen secretion.

Androstenedione, DHEA and 17α-hydroxyprogesterone are thought to originate both from the suprarenals (zona reticularis) and the testes. These substances are hormones as well as precursors in the biosynthesis of testosterone and cortisol (35, 36). If the adrenal androgens follow the same response pattern as cortisol and DHEA-S, and the testicular androgens follow the same response pattern as testosterone, then most (90%) of the circulating 17α-hydroxyprogesterone, DHEA and androstenedione have to be of testicular origin. However, it has been shown that only 10–25% of the circulating DHEA, 25–30% of the androstenedione and 10% of the circulating 17α-hydroxyprogesterone derive from direct testicular secretion (34). This indicates a differential regulation of the various adrenal steroids.

Adrenocorticotropin (ACTH) is known to stimulate all adrenal hormones but most efficiently the cortisol production. It has been speculated whether β-endorphin might stimulate selectively the zona reticularis to produce androgens. However, both ACTH and β-endorphin are known to increase during stress and should therefore have given the opposite results. Several researchers believe that a specific tropic hormone, probably distinct from those presently recognized and probably of pituitary origin, is uniquely responsible for adrenal androgen production. This hormone has been termed adrenal androgen-stimulating hormone (AASH) or cortical androgen-stimulating hormone (CASH) (34, 37–39). Such hormones may be involved in the increased cortisol secretion and decreased adrenal androgen secretion during the course, as well as the altered circadian rhythm.

Normally DHEA-S and DHEA are interconverted mainly in the liver but also in other peripheral tissues. It is interesting to note that during the present course there is an increase in DHEA-S simultaneously with a decrease in DHEA in serum, indicating a differential regulation of these two hormones. These alterations may be explained by the different adrenal secretion rates of the sulfated and non-sulfated DHEA during the course, and also by an inhibition of the desulfation mechanism or a stimulation of the sulfokinase acting upon circulating DHEA.

The androgenic effects of DHEA-S are very weak. However, owing to its very high concentration in plasma, this hormone may compensate partially for the decreased levels of non-sulfated androgenic hormones. During the ranger training course the sexual hair growth (beard) is gradually reduced during the first day of activities and is almost absent at the end of the course. The total beard growth during the 5 days corresponds to 1–2 days of normal growth. This indicates that there is a real decrease in the androgenic functions during the course and that DHEA-S is not able

to compensate for the decrease in the other androgenic hormones.

No circadian rhythm in plasma catecholamines was observed in the control experiment or during the course. This contrasts with other investigations, which have shown a circadian rhythm of catecholamine excretion in urine as well as in plasma levels, with an increase during daytime and a decrease during night-time (14, 18, 19, 40, 41). The circadian rhythm for urinary catecholamines may be explained by the diurinal alterations in the urine excretion (42). In addition the catecholamine secretion is influenced by several external stimuli, such as exercise, energy intake, salt intake, cold, psychological stress, sleep disturbances, etc., which might influence the plasma levels as well as the urinary excretion of catecholamines. Some investigators have tried to establish the endogenous circadian rhythm of catecholamines by reducing all possible external influences on the catecholamine secretion, i.e. by keeping the subjects on a strict regimen and supine in bed (13, 15) or with only light physical activities (16). Even supine in bed the catecholamine secretion is probably influenced by the circadian rhythm of general activation, sleep/wakefulness, body temperature, etc. During the present control and recovery experiment the cadets were allowed to sleep during the night-time but had to wake up and walk slowly for 2–3 min to the area where the blood samples were drawn. However, before the blood sampling they were tested for mental performance for about 15 min. The cadets then were allowed to sit quietly on a chair for 10–15 min before blood sampling. In this way the cadets had approximately the same type and degree of activities in the hour preceding the blood sampling. In this situation there was no circadian rhythm for plasma catecholamines, which is in accordance with the results of Cameron et al. (13), who found no circadian rhythm when the subjects were supine in bed during the whole experiment.

During the first day of activities the catecholamines increased gradually. At the end of the course the noradrenaline levels were still high, whereas adrenaline and dopamine had decreased. This indicates an increased activation of the sympathetic nervous system and a decreased significance for the adrenal medulla at the end of the course.

Plasma glucose was not changed significantly during the course and no circadian rhythm was found. The variations observed probably were due to preceding food intake and glucose intolerance rather than to the effect of the circadian rhythm.

Human growth hormone is known to increase during sleep (12). No significant circadian variations were seen during this experiment. The increases observed probably are due to preceding physical activities, which are known to stimulate hGH.

Thyroid-stimulating hormone displayed a circadian rhythm with the highest level at about midnight and the lowest level in the afternoon, both in the control experiment and during recovery, whereas no similar rhythm was found for thyroid hormones. This indicates a circadian rhythm in the thyroid sensitivity for TSH. In contrast, Weeke and Gundersen (23) found a small diurnal variation in T_3 and FT_3 that was related temporally to the variations in TSH, whereas Nimalasuriya et al. (43) concluded that the diurnal rhythm found for T_3 is regulated by some dietary signal, which alters the efficiency of the peripheral tissue T_4-to-T_3 conversion and is independent of TSH.

The increase in T_4, FT_4 and T_3 during the first day of activities reached its maximum already after 12 h of activities and was not due to TSH stimulation because the TSH level decreased simultaneously. During a previous course we found that TSH decreased most in the subjects who were allowed 3 h of sleep each night and least in the subjects who were given extra food (25). This is in accordance with other investigators, who have shown that sleep inhibits TSH secretion (22). In spite of this, the circadian rhythm of TSH was abolished at the end of the course but re-established after 4–5 days of recovery. The decreased plasma levels of thyroid hormones were normalized after 4–5 days of recovery for T_4 and FT_4 but not for T_3, FT_3 and TSH. In accordance with previous investigations (25), the plasma levels of T_4 and T_3 were lower in the morning of day 1 than 1 week prior to the course. This is probably due to the lower T_4-binding globulin levels on day 1 than normally. The dissociation between the increase in total T_3 and FT_3 may also be explained by a simultaneous increase in T_4-binding globulin. Others have found a small diurnal variation in thyroid hormones.

For both mental performance and hormones there was a strong correlation between the levels at different time points. This shows that the relative time-to-time alterations were similar in the different cadets.

In conclusion, this paper has shown that during prolonged continuous stress there is a dissociation between the amplitude of the circadian rhythm for mental performance, which is increased, and for plasma steroids, which is extinguished. The circadian rhythms for plasma cortisol and DHEA-S were extinguished above the maximum level, and for the androgens below the minimum level observed in the control experiment.

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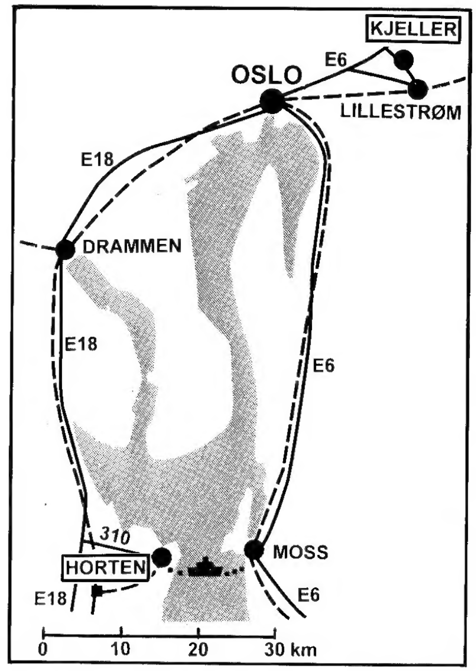

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Publications from NDRE:

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